The influence of sympathetic failure on the skin microcirculation of the diabetic neuropathic foot.

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Niet voor mijn eega,

wel voor mijn ego.

Contents

Chapter 1

Introduction and outline of the study

Introduction

A major burden both to the diabetic patient and to the health care system are foot disorders. The possibility of amputation is a life long threat to the diabetic patient. Foot problems cause more inpatient bed occupancy than all other diabetic medical complications together [1,2].

Although the understanding of the pathogenesis of diabetic foot problems has progressively increased in recent years, diabetes still accounted for 46% of the 3342 lower extremity amputations in the Netherlands in 1991. Total, age and sex adjusted incidence per 10.000 diabetic patients of lower extremity amputation was 23.7 as compared with 1.3 in the non-diabetic population [3].

The diabetic foot can be classified into the neuropathic foot and ischaemic foot. The neuropathic foot is characterized by painless neuropathic ulcers, Charcot joints and neuropathic oedema. In the ischaemic foot atherosclerosis is the dominant factor leading to a reduction in blood flow with absent pulses. Combining the results of several studies, neuropathy can be considered as the major etiological factor in 90% of more than 600 episodes of diabetic foot ulceration [4,5]. Therefore the diabetic foot appears to be mainly neuropathic in origin [6].

The most common type of neuropathy associated with diabetes mellitus is distal symmetrical sensory polyneuropathy, often accompanied by autonomic neuropathy [7]. The genesis of the neuropathic ulcer is not yet fully understood. Loss of sensation, especially awareness of pain is required, but there is evidence from studies on dogs that somatic denervation alone is insufficient to cause ulceration, and that sympathetic denervation is also necessary [8,9].

A common observation in diabetic neuropathy is a warm leg and foot, with often easily palpable and visible pulses of the foot arteries, especially in the dependent position [10]. Nevertheless, within centimetres of such an apparently adequate blood supply there may be a penetrating neuropathic ulcer.

Several studies suppose that total skin blood flow in diabetic neuropathy is increased, as a result of an increase in shunt flow through sympathetic denervated arteriovenous anastomoses (AVA). A study using radiolabelled microspheres demonstrated that vascu-

lar channels of greater than 20 μm diameter are present in the feet of subjects with neuropathic ulcers [11]. These were assumed to be dilated AVA, which can reach 60 μm in diameter. In diabetic patients with a neuropathic foot ulcer, Doppler ultrasound studies of proximal arteries showed an increased flow, with a greater than normal forward systolic flow, accompanied by a loss of diastolic back flow, suggesting rapid shunting [12,13]. Further indirect evidence was obtained by measuring a high partial oxygen pressure in the foot veins of patients with diabetic neuropathy, suggesting that saturated blood passes directly into the venous circulation [14]. Several studies in diabetic neuropathy using venous occlusion plethysmography [15] and laser Doppler fluxmetry [16] have demonstrated increased skin blood flow in the toe pulp, a site where AVA are numerous.

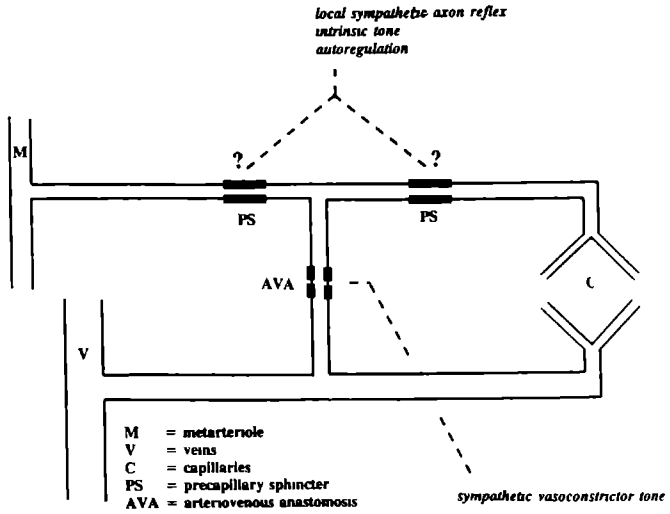

Fig 1 **Diagrammatic representation of skin microcirculation**

Beside the AVA, skin microcirculation is made up of another major system, the superficially located capillaries (Fig. 1.). While the capillaries serve nutritional demands and are important in the process of wound healing, the AVA primarily have a thermoregulatory function. The anastomoses are controlled by sympathetic nerve endings, in contrast to the capillaries, which have no nerve supply [17]. Under conditions of normal environmental temperature the majority (80 - 90%) of skin blood flow passes through the anastomoses. Sympathetic stimulation, for example a cold stimulus, results in vasoconstriction of the anastomoses, reducing total skin blood flow tremendously [18]. On the contrary under pathological circumstances for example after sympathetic denervation, baseline skin blood flow increases and sympathetic stimulation does not induce a decrease in skin blood flow. [19].

Skin microcirculatory inflow is regulated by precapillary sphincters (Fig. 1.). By lowering the limb, venous hydrostatic pressure increases, which results in vasoconstriction of the precapillary sphincters. This postural vasoconstriction response is probably mediated either by the sympathetic nervous system or by local neurogenic or myogenic mechanisms [20]. In diabetic patients with neuropathy, the postural vasoconstriction response may be defective and may explain the red warm foot skin, which is most prominent in the dependent position [21].

Despite an increase in peripheral skin blood flow in diabetic neuropathy, ulceration may co-exist. As a possible explanation, a capillary steal phenomenon has been postulated. In this hypothesis, the increase in AVA flow reduces nutritive capillary flow [22], resulting in skin ischaemia [23].

To test this hypothesis several tools are necessary. First, adequate methods should be available to select diabetic patients with peripheral (autonomic) neuropathy and to quantify its severity carefully. Secondly, instruments to measure nutritive capillary blood flow and AVA flow separately. Thirdly sensitive and specific tests to study sympathetic vasomotor reflexes. Fourthly, three well-defined and sex and age matched study groups of diabetic patients, one with and one without neuropathy and a third group of healthy controls will be necessary, all without macro-angiopathy.

Therefore several studies had to be performed before the final skin microcirculatory study in diabetic patients with well-defined neuropathy could be done, to support or reject the supposition of a capillary steal phenomenon.

Ad 1. In the literature uniform, internationally accepted criteria for the diagnosis of diabetic neuropathy are lacking. Therefore, several neurological symptom scoring lists, nerve conduction tests, quantitative sensory examination, as well as quantitative autonomic examination are recommended [24]. Assessment of nerve conduction provide the most objective, specific, sensitive and repeatable method for the detection of nerve dysfunction, but disturbances do not directly reflect symptoms or neuropathological lesions [25]. Many techniques are available for quantitative sensory examination, but most are poorly standardized and reproducible while normal values have not been defined. Therefore, each laboratory should standardize these techniques by using their own population norms and should report both the absolute data and the relationship of the data to the appropriate normative control population [24].

Ad 2. Capillary microscopy is a useful method that allows direct visual access to the nutritive capillaries. Most often the nailfolds are examined, because of accessibility and optimal capillary visualization. At the nailfold edge the papillae, which shelter the capillary loops are oriented parallel to the skin surface. For measurement of capillary blood cell velocity (CBV) of the human skin, a video-photometric, cross-correlation technique was developed by Intaglietta and coworkers [26]. A microscope-television system enables video-recording of capillary dynamics. Thereafter the video-signal passes through a photometric analyzer that generates two windows onto the TV-screen over a capillary loop. These windows are sensitive to variations in the light intensity within the window. Passage of red blood cells, leucocytes and plasma gaps through the investigated capillary loop causes variation in optical density, which are quantified by the windows and converted to electronic equivalents. By determining the time interval by which the distal signal of the second window has to be delayed to achieve maximum cross-correlation with the proximal first window signal, the CBV is calculated [27]. With a fully computerized system (CapiFlow^{R}, IM-CapiFlow, Stockholm, Sweden), the whole pro-

cess of CBV calculations can be performed both continuously and automatically.
AVA blood flow can be measured by laser Doppler fluxmetry (LDF). The LDF-meter uses a low power laser (usually helium neon 2-3 mW) to generate a non-injurious beam of infrared light which passes through an optical fibre to illuminate a region of skin tissue. Photons entering the tissue are scattered by moving red blood cells and undergo a frequency shift according to the Doppler principle. A portion of the reflected light is collected and delivered via other optical fibres to a photodetector whose electrical output is processed to yield a continuous reading which is proportional to the local blood flow. Approximately 40% of the light from a He-Ne laser penetrates to a depth of 0.5 mm and only 5% reaches 1.77 mm [28]. The AVA are located on a depth of 0.5 - 1.0 mm. Consequently, the resulting back-scattered laser light signal partly comes from the arteriovenous blood flow. In agreement with this conclusion, synchronous assessment of human skin microcirculation by LDF and capillaroscopy showed discrepancies, which can be interpreted as evidence that LDF records blood flow in deeper located vessels in addition to the superficial capillaries [29]. Therefore LDF can be used to monitor variation in AVA flow.

Ad 3. LDF can be used to evaluate skin vasoconstrictor responses [30]. Sympathetic stimulation e.g., a cold stimulus, the Valsalva manoeuvre and an inspiratory gasp will result in a decrease of LDF. Because of difference in baseline sympathetic tone and probably age-dependency [31], there is a great variability in the LDF response during sympathetic stimulation [30,32]. Furthermore skin blood flow not exclusively depends on sympathetic innervation [33]. Beside the blood cells, all moving objects of a certain structure will give rise to the LDF signal and the measuring volume and penetration depth are not fixed, but vary with the amount of blood and the composition of the skin in the region investigated [34].
Therefore optimal conditions to standardize baseline sympathetic tone should be evaluated and the usefulness of the different skin vasomotor reflex tests measured with LDF should be analyzed.
Recently it was claimed that a newly developed LDF device, where the source, a small diode laser, and detector are integrated in the probe, may have less spatial and temporal

variability of the LDF measurements [35]. This is the result of a decreased sensitivity to movement artifacts, differences in geometry of the detectors, wave-length of the laser light and a higher frequency of the low-pass filter. The diode laser will be compared with the commonly used Ne-He laser, to evaluate reproducibility of the LDF measurements with both instruments.

Ad 4. For careful selection of diabetic patients with and without neuropathy, the out-patient clinic of the University Hospital Nijmegen had to be screened. To collect all the selected data, several computer programs are available, but they are too extensive and often not enough focused to diabetic foot problems.

In conclusion; the principle aim of our study was whether a capillary steal phenomenon exists in the foot skin microcirculation of diabetic patients with neuropathy.
As described, different prerequisites had to be prepared before the main question could be resolved in an adequate protocol. Therefore in chapter 2 and 3 methods are described to quantify diabetic polyneuropathy and autonomic neuropathy. Evaluation of sympathetic skin vasomotor reflexes with LDF devices are reported in chapter 4 to 6. The influence of temporary nerve denervation on arteriovenous shunt flow and capillary blood flow is tested in a model that simulates neuropathy. The results of this study is reported in chapter 7. In chapter 8 the results of a computerized survey of the diabetic university out-patient clinic are reported, which special emphasis on diabetic foot problems. With this database, patients were selected for the foot skin microcirculation study of chapter 9 to test the capillary steal hypothesis.

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Chapter 2

Comparison of clinical examination, current and vibratory perception threshold in diabetic polyneuropathy

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Abstract

The study of diabetic polyneuropathy is complicated by a lack of clear definitions and the absence of a simple reliable test procedure. Recently, a new sensory perception testing device has been introduced for detection of thresholds for electrical stimuli (current perception: CPT) at different frequencies (Neurometer^{R}). We compared standardized clinical examination scores with measurements of vibratory perception threshold (VPT) and CPT (foot) and obtained reproducibility figures.

Participants in the study were healthy controls (H, $n = 33$), diabetic patient without clinical signs of neuropathy (DN -, $n = 23$), diabetics with overt diabetic neuropathy (DN +, $n = 22$), and patients with a diabetes duration of over 20 years (D20, $n = 38$).

As expected, there were highly significant differences (Wilcoxon) in CPT, VPT and neurological scores between H / DN - and DN + ($p < 0.001$), but not between DN - and H.

Correlation between CPT and total as well as partial (reflecting small and large fibre functions) neurological examination score were highest at 2000 Hz ($r = 0.88$); no advantage of lower frequency CPT could be identified. CPT seemed rather insensitive in detecting neuropathy. Correlations between CPT and VPT were only moderate and maximal at 2000 Hz ($r = 0.61$).

Reproducibility of CPT was good at 2000 Hz (coefficient of variation 13.3 - 20.2 %), but moderate to poor at lower frequencies (ranging to 62%).

We concluded that CPT and VPT quantitative sensory testing is only of limited value, mainly because of high variability and poor reproducibility.

Introduction

A wide variety of peripheral nerve dysfunctions can occur in relation to diabetes mellitus [1] Of these, symmetric sensory polyneuropathy is the most common complication of long-standing hyperglycaemia [2], and an important factor in the etiology of foot ulceration [3,4]

The study of diabetic neuropathy is complicated, however, by the lack of a clear definition and the absence of a simple, universally accepted, test procedure [5] Measurement of nerve conduction velocity is an established method to quantify peripheral nerve function [2] Although abnormality of nerve conduction is objective, specific, sensitive and reproducible [6], and although test results correlate with clinical neuropathy [7], disturbances do not directly reflect symptoms or neuropathological lesions [7-9] Moreover, results depend on ambient blood glucose value and temperature of limbs [2] Considerable skill and experience on the part of the investigator are necessary In fact, nerve conduction measurements only refer to large myelinated fibres Finally, the technique can be uncomfortable for the patient and is time-consuming

Several devices have been developed to test somatosensory nerve functions These methods are simple, non-invasive and capable of examining the function of nerve fibres, which is not easily accessible to electrophysiological evaluation [10] By this, they may correlate better with clinical signs These "psychophysical" assessments of sensory perception thresholds can be performed in a variety of ways Devices producing graded vibratory stimuli are used for many years [11,12] and are generally accepted as an objective assessment of nerve function [13] Recently a new device has been introduced for the measurement of sensory perception thresholds for electrical stimuli at varying frequencies (Neurometer^{R}) The producers claim that selective stimulation of three subsets of nerve fibres is provided Initial reports seemed to confirm these claims [14] Our study was undertaken therefore to determine whether sensory testing, especially with the Neurometer^{R}, is able to detect, quantify and characterise diabetic neuropathy in a reliable way A systematic, detailed, clinical examination, which was chosen as standard, was compared with measurements of current and vibratory perception threshold Also, reproducibility figures were obtained

Patients and methods

Subjects

Four groups of participants were selected: (1) healthy controls (H, mainly consisting of hospital staff and family, $n = 33$); (2) diabetic patients without any clinical sign of neuropathy (DN-, $n = 23$), defined as absence of symptoms or signs; (3) diabetic patients with overt diabetic neuropathy (DN+, $n = 22$), carefully selected on the existence of at least 2 out of 5 symptoms or signs (pain, paraesthesia, numbness, absence of reflexes and disturbed vibration perception tested by 128-Hz tuning fork); and (4) diabetic patients not selected on neurological conditions but with a known duration of diabetes mellitus of 20 years or more (D20, $n = 38$).

All participants met the exclusion criteria: renal failure (serum creatinine > 120 μmol/l) and liver disease (enzymes above twice normal value), concomitant neurologic disease or passed trauma with persisting disturbances of the peripheral nervous system, other metabolic or endocrine diseases besides diabetes mellitus, peripheral vascular insufficiency (normal palpable foot pulses, absence of trophic skin alterations and normal capillary refill), alcohol intake $\geq$ 4 units/day, vitamin B_{12}- and/or folate-deficiency, medication consisting of analgetics, phenytoin and/or phenothiazines, known contact with neurotoxins. All patients were under 70 year and had diabetes type I or II.

Measurements

Clinical examination A scoring system for symptoms and clinical evaluation modified from reported general neurologic scoring systems [7,15] was developed. Sensory qualities and investigation of the lower extremities were emphasized and a limited number of scoring categories was chosen to improve reproducibility [10,16]. At a recent consensus meeting the use of graded scales in clinical measures was again emphasized [17].

Thus, after inclusion, a *neuropathy symptom score* [7], was obtained. Items were considered positive (1 point) if a symptom existed for at least 30 days; the maximal score was 14 points (Table 1 lists the 14 questions of the modified neuropathy symptom score).

Then, a standardized neurological examination was performed based on a modified *neuropathy disability score* [15]. Modification consisted of extension of sensibility

testing at the lower extremities, of simplifying the reflex examination, and of omitting of investigation of cranial nerves, muscle strength and sensibility at upper extremities. The

Table 1. **Modified Neurological Symptom Score**

1. Muscle weakness shoulder/upper arms
2. Muscle weakness hands
3. Muscle weakness glutei and thigh
4. Muscle weakness legs
5. Difficulties in identifying objects in mouth
6. Difficulties in swallowing
7. Difficulties in identifying objects in hands
8. Unsteadiness in walking
9. Paraesthesia
10. Numbness
11. Pain - burning, deep aching, tenderness
12. Postural fainting
13. Loss of urinary control
14. Night diarrhoea

Normal 0 points, if symptoms exist for ≥ 1 month: 1 point. Maximum-score 14 points

score was simplified to three possibilities: no disturbances, 0 points; slight or moderate disturbances, 1 point; severe disturbances/absence, 2 points. Both sides of the body were scored; the maximal score was 48 points (Table 2 lists the tested items in this modified neurologic disability score). All tests were performed by two investigators (M.S. and J.W.M.). The inter-observer reproducibility of this scoring system was 26.8% in a subgroup of 12 patients (of group 3, DN+, clinical overt neuropathy). Others also reported a favourable reproducibility and good agreement of standardized neurologic examination [18], while recently, in a UK nationwide study a very similar scoring system was used [19].

Current Perception Threshold (CPT) CPT's were obtained using a commercially available device (Neurometer^{R}, Minimed Technologies, Sylmar, CA, USA), which has been described previously in the literature [21]. This portable, battery-operated (6 V) device

generates a graded sinusoid at 5, 250 and 2000 Hz with a constant current from 0 to 10 mA. The current is delivered to the skin surface via a pair of gold electrodes 1 cm in diameter separated by 1.7 cm and covered by a thin layer of electrode paste. The three

Table 2. **Modified Neurological Disability Score**

Part I: REFLEXES	
	Quadriceps femoris
	Triceps surae
Part II: TESTING OF GNOSTIC SENSITIVITY	
Fine touch pressure :	Lower leg
	Dorsum of foot
	Large toe
Vibration (tuning fork):	Patella
	Medial malleolus
	MTP-joint
Joint position sense:	Large toe
Part III: TESTING OF VITAL SENSITIVITY	
Pricking pain sense:	Lower leg
	Dorsum foot
	Large toe

Normal: 0 points. Mild/moderate deficit: 1 point. Severe deficit/complete absence: 2 points. Both sides scored. Maximum score: 48 points.

frequencies are thought to be selective in their physiological excitation of type A (2000 Hz), B (250 Hz) and C (5 Hz) afferent fibers. First intensity of the stimulus is increased until the subject is able to distinguish the sensation. Subsequently the lowest level (threshold) of electrical stimulus that an individual is able to perceive approximately half of the time is determined by delivering a range of intensities of which the tested person has to choose whether he feels the stimulus or not (forced choice method). This pro-

cedure is repeated for each frequency. CPT's were obtained at the finger (median nerve) of the dominant side, (groups 1, 2 and 3) and great toe (peroneal nerve) of the dominant side (all groups).
Fifteen persons of group 1, 15 of group 2, 14 of group 3 and 19 of group 4 were re-assessed after 1-30 days. (All patients were asked for a second measurement but due to logistic reasons and refusal this was not possible in all). Twelve patients of group 3 were reinvestigated after 3-4 months by a second observer.

Vibratory perception threshold (VPT) VPT's were obtained at the metatarsal phalangeal (MTP) joint in subjects of group 1, group 3 and 4, and repeated in 19 patients of group 4, using a Somedic Vibrameter IV in which the pressure with which the stimulator is applied to the test site is standardized [22]. With this device an increasing stimulus was applied until the tested individual perceived the vibration. The intensity was then decreased until the subject stated that he/she no longer perceived the stimulus. This procedure was repeated twice. The mean of the 6 values obtained was taken as VPT. Since vibratory threshold is age-dependent [12,23] and can be described by:

$$\log(V_T) = a + b * A$$

where V_T = Vibratory threshold in micrometers, A = Age and a en b represent known constants for each investigation site, the values obtained were recalculated to a standard age.

Statistical analysis

Overall statistical analysis was performed using non-parametric one way analysis of variance (Kruskal-Wallis). Subsequently, differences in groups were evaluated by unpaired Wilcoxon test. Spearman's rank correlation coefficients were used for equation of correlations. Standard error of a single observation was calculated according to the formula:

$$\text{SESO} = \sqrt{\sum_{i=1}^{n} \frac{(X1_i - X2_i)^2}{2n}}$$

X_i = result of the first test and Y_i = result of the second test of subject i, n = number of paired observations, i = 1 to n, for reproducibility figures, and expressed as a percentage of the mean value of the first test. This method of expression takes into account all individual values. The lower the percentage, the better the reproducibility.

Results

Clinical characteristics of the groups are given in table 3. Non-parametric ANOVA revealed p - values of < 0.002 for CPT, VPT as well as neurological symptoms score, indicating significant differences between the groups.

Distributions of individual CPT's obtained at the large toe at 2000 Hz are shown in Fig 1. There was no statistical significant difference between results of CPT testing in normals (H) and DN- ($p = 0.70$). Highly significant differences were found between H and DN+ ($p < 0.001$), between DN- and DN+ ($p < 0.001$) and between D20 and DN+ ($p < 0.01$). Further, a significant discrimination was established between H and DN- on the one side and D20+ on the other side ($p < 0.02$). Among DN+ and D20 patients a considerable number were unable to perceive even the highest stimulus (9.99 mA).

Distribution of individual CPT's at 5 and 250 Hz were very much alike (not shown), although generally values for CPT 250 Hz were lower and for CPT 5 Hz lowest. Thus, at 5 and 250 Hz again highly significant differences were identified between H and DN+, DN- and DN+ and between D20 and DN+ (all $p < 0.01$), while no difference was found between H and DN-. With these frequencies however, no difference between H/DN- and D20 could be established.

Despite significant differences between the groups there is a wide overlap of individual values, as shown by Fig 1.

Table 3. **Clinical characteristics of the four groups** (Means ± SD are given).

	1. Control	2. DN-	3. DN+	4. D20
Number	33	23	22	38
♂ : ♀	18 : 15	10 : 13	10 : 12	22 : 16
Age (year)	33.1 (±14.2)	32.6 (±14.4)	53.2 (±12.9)**	44.3 (±12.6)
Quetelet-index (kg/m^2)	22.6 (±2.5)	23.7 (±3.1)	26.3 (±5.9)**	24.5 (±3.5)
Diabetes duration (yrs)		14.5 (±9.0)	24.0 (±7.5)*	27.2 (±8.2)
HbA_{1c} (%)#		9.3 (±1.8)	9.5 (±1.6)	9.0 (±1.6)
DM type I : II		20 : 3	15 : 7	33 : 5
Number of patients with:				
Retinopathy (proliferative)		6 (0)	20 (5)	19 (4)
Nephropathy (micro-albuminuria)		2 (1)	10 (3)	12 (6)
Macroangiopathy		0	8	4

*** $p < 0.01$, * $p < 0.05$ compared to group 2 (DN-). # Reference value 4.2 - 6.3%. Retinopathy: past treatment with laser photocoagulation by ophthalmologist. Between brackets the number with proliferative retinopathy. Nephropathy: albuminuria 30 - 300 µg/24 hr (micro-albuminuria, "incipient nephropathy"; between brackets) or > 300 µg/24 hr (macro-albuminuria " overt nephropathy"), in combination with retinopathy. Macroangiopathy: overt manifestations of vascular disease (coronary heart disease, passed cerebrovascular accident); peripheral vascular disease already excluded.*

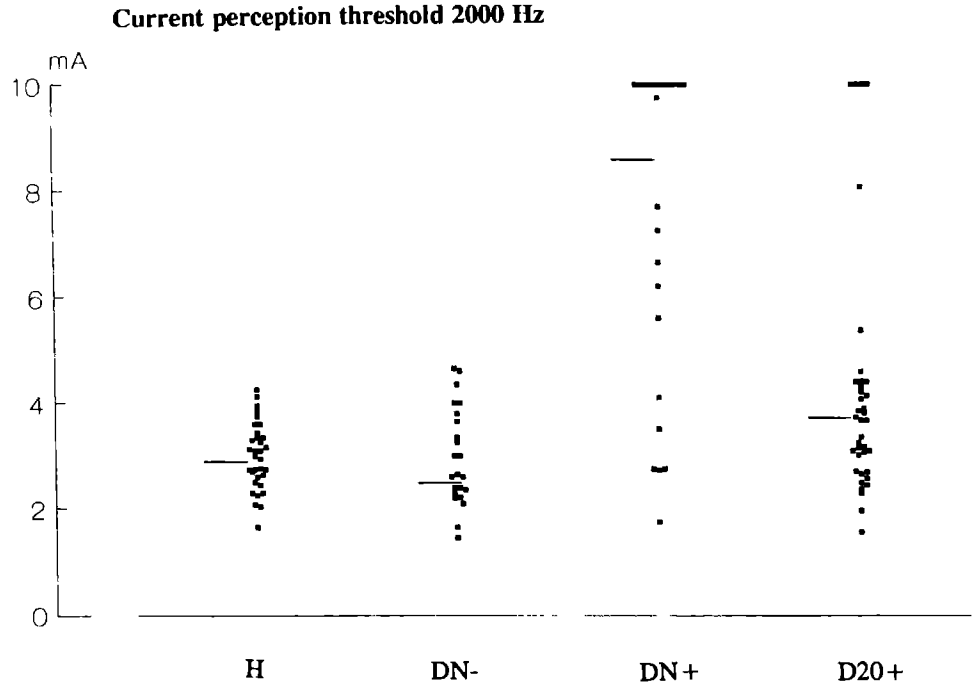

Fig. 1. **Individual and median values of CPT 2000 Hz in H (healthy), DN- (no clinical signs of neuropathy), DN+ (clinical signs of neuropathy) and D20 (over 20 years diabetes).** CPT given as μA. Differences: H vs. DN-: $p < 0.001$; H vs. DN+, $p < 0.001$; H vs D20, $p < 0.02$; DN- vs DN+, $p < 0.001$; D20 vs DN+, $p < 0.01$; D20 vs DN-, $p < 0.02$.

CPT's obtained at the finger did not show significant differences between H, DN- and DN+.

Individual values of VPT tests are shown in Fig 2. All groups were significantly different from each other, not only H versus DN+ ($p < 0.01$) and D20 versus DN+ ($p < 0.02$) but also H versus D20 ($p < 0.05$). Total neurological examination scores as demonstrated in Fig. 3 were different between DN- and DN+, D20 and DN+, and DN- and D20 (all $p < 0.001$).

Correlations between score at neurological examination and different frequencies of CPT as well as VPT obtained in D20 are listed in Table 4. Correlation with symptoms was weak (data not shown) and worse than with neurological signs.

Correlations between the two sensory test procedures obtained in DN+ and D20 are also shown in Table 4. Correlations were generally poor; best correlation was found between CPT at 2000 Hz and VPT ($r = 0.61$, $p < 0.05$).

In the group selected on clinical signs of neuropathy (DN+) correlations between

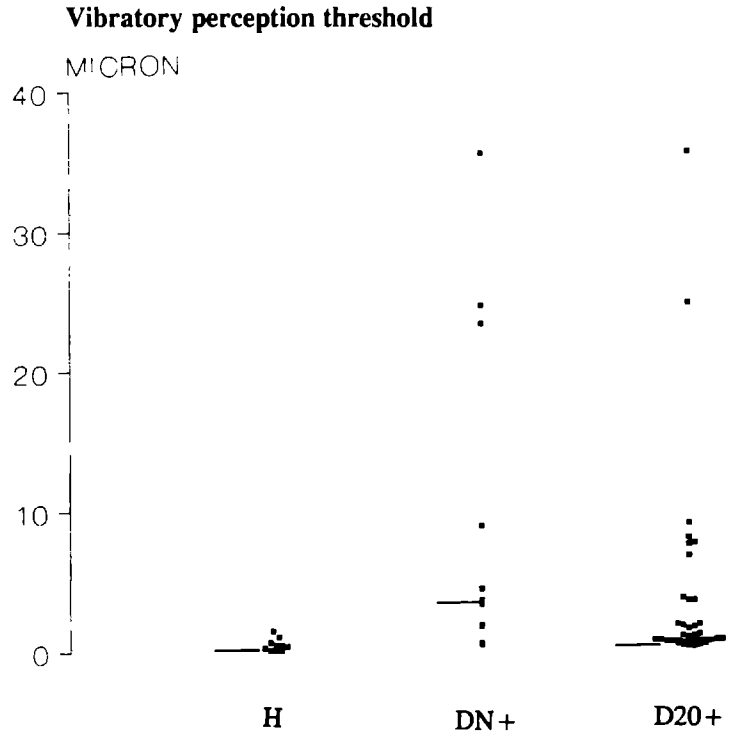

Fig. 2. **Individual and median values of VPT obtained in H (healthy), DN+ (clinical neuropathy) and D20 (over 20 years diabetes).** VPT given as μm. Differences: H vs DN+, $p < 0.01$, D20 vs DN+: $p < 0.02$, D20 vs H: $p < 0.05$.

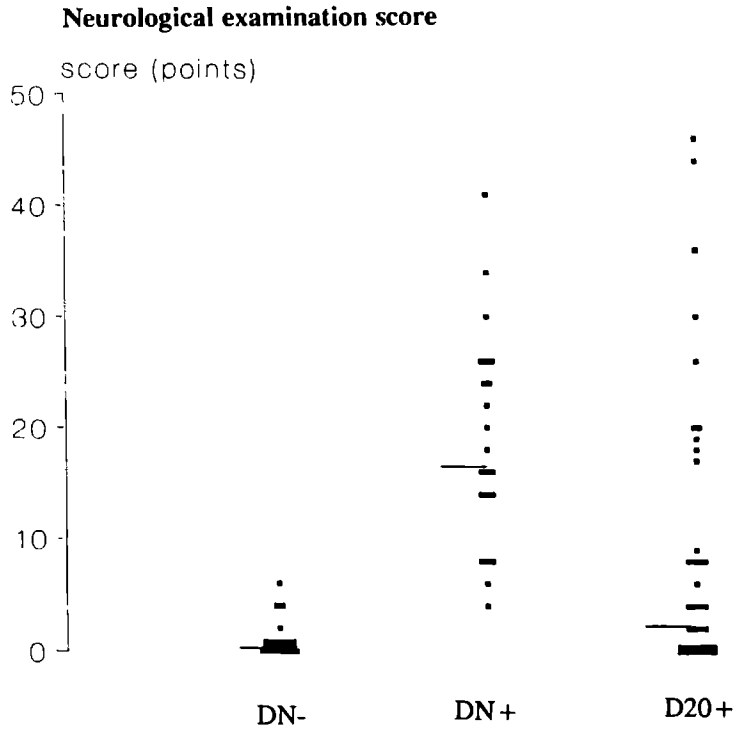

Fig. 3. **Individual and median values of total scores of modified neurological disability score obtained in DN- (no clinical signs of neuropathy), DN+ (clinical neuropathy) and D20 (over 20 years diabetes).** Values expressed in points. Differences: DN- vs DN+: $p < 0.001$; D20 vs DN+, $p < 0.001$; DN- vs D20, $p < 0.001$.

perception thresholds and different parts of physical examination were calculated to detect differentiations of test results and small and large fibre functions (Table 5). The highest correlations were found between various parameters and CPT at 2000 Hz. There was however no obvious differentiation in high and low frequency CPT on the one side and tests of large and small fibre function on the other.

In the group not selected for neurological signs (D20) a neurological score $\geq$ 2 points

Table 4. **Correlations between CPT, VPT and examination score**

	Disability score	Vibration perception threshold	
	Group 4 (D20, n=38)	Group 4 (D20, n = 38)	Group 3 (DN+, n=12)
CURRENT PERCEPTION THRESHOLD			
2000 Hz	0.68***	0.52**	0.61*
250 Hz	0.58***	0.52**	0.49
5 Hz	0.53**	0.56***	0.52
VIBRATORY PERCEPTION THRESHOLD			
MTP-joint	0.73***		

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.002$. *CPT's were obtained at the great toe of the dominant side.*

Table 5. **Correlations between examination score and perception thresholds**

	NDS reflexes	NDS large fibre	NDS small fibre	NDS total
CURRENT PERCEPTION THRESHOLD				
2000 Hz	0.64*	0.88***	0.86***	0.88***
250 Hz	0.57*	0.74**	0.72*+	0.75**
5 Hz	0.47	0.69*+	0.74**	0.69*+
VIBRATORY PERCEPTION THRESHOLD				
MTP-joint	0.42	0.77**	0.63*	0.59*

* $p < 0.05$, *+ $p < 0.02$, ** $p < 0.01$, *** $p < 0.002$.
NDS = modified neurological disability score (see text and Table 2 for explanation). "NDS reflexes" stands for score on part I, large fibre for sensibility-score of part II (fine touch, vibration sense and joint position sense), "NDS small fibre" for score of pain sense (part III).
Current perception threshold was obtained at the great toe of the dominant side.

existed in 53%, of ≥ 4 points in 42% of the patients. VPT ≥ 2 SD above normal was found in 55%, and CPT ≥ 2 SD in only 26% (10 patients, 9 of whom were also abnormal at VPT examination).

Reproducibility was best in CPT at 2000 Hz as can be seen in Table 6. Reproducibility was poorer in the lower CPT frequencies, especially in the group with deficits. In a subgroup of DN+ intra-individual reproducibility was better than inter-individual repeatability.

Discussion

The main finding of our study is the fact that sensory testing shows high variability and poor reproducibility while systematic standardized examination seems fairly discriminative and reproducible.

Assessment of diabetic neuropathy still is a problem due to lack of clear definitions and standardized evaluative procedures [5,24]. Therefore, to fully classify diabetic neuropathy, measurement of clinical symptoms, clinical examination, electrodiagnostic studies, quantitative sensory testing and autonomic function testing are recommended [10]. In clinical practice this, however, this is not easily done. Full characterisation is probably only necessary in clinical research settings [10].

Because of relative simplicity and presumed objectivity and reliability, sensory perception testing is an attractive alternative for detection and quantification of diabetic neuropathy. Many devices are available for objective measurement of sensory perception and new ones continue to be introduced. One of the latest developments is CPT testing with a variable constant current sine wave stimulator (Neurometer^{R}). Despite the proposed advantages [21] the intrinsic problems of sensory perception testing cannot be excluded. These are the need of for the full cooperation and attention of the patient and thus possible variability due to psychological factors as lack of motivation, easy distractibility, fatigue and poor comprehension [25].

Groups were defined by very strict criteria as with or without neuropathy; in doubt, cases were excluded. Because patients with only slight disturbances were probably not

included, a fourth group was formed not selected on neurological disorders but with a known diabetes duration of over 20 years (After 25 years a prevalence of about 50% [26] would be expected.) Scores of neurological examination turned out to be highly significant different between this group (D20+) and DN- and DN+ The same applied to CPT testing at 2000 Hz and VPT testing, but CPT 250 and 5 Hz could not discriminate between a group with high prevalence ($\approx$50%) of neuropathy and healthy controls.

In determining prevalence of nerve dysfunction, CPT testing also turned out to be relative insensitive, identifying only half of the patients who were, based on other examinations, probably affected by neuropathy VPT testing seemed in this regard to be as sensitive as neurological examination score

It has been suggested that CPT testing at different frequencies creates the ability to stimulate different types of nerve fibres, but when comparing results of lower frequencies with pain sensation (an established small fibre function with neuropathological changes different from large fibre involvement [27]), no advantage of lower frequencies could be found. In fact, correlation was best and high between all aspects of neurological investigation and CPT at 2000 Hz Also, in a recent publication, no correlation between CPT (or VPT) and morphometric findings of sural nerve biopsy could be identified [20]. Temperature discrimination is also a known index of small fibre function [28]. Although correlations between disturbed thermal threshold and painful neuropathy have been described [29], reproducibility is extremely poor [16,23], especially in patients with established nerve dysfunction. Masson and colleagues found a significant correlation between CPT at 5 Hz and thermal threshold testing, but the correlation coefficient was only 0 34 [14].

Because vibration sense is a function of large myelinated nerve fibres [2], correlation of VPT was, as expected best with large nerve fibre function

Correlation of symptom score with quantitative sensory thresholds is low; it is generally known that symmetric neuropathy with risk of foot ulceration is often symptomless [1]. The low correlation between two investigations presumed to measure the same quality probably reflects the variability of these measurements.

Reproducibility of CPT testing was best at 2000 Hz and moderate at lower frequencies

Table 6. **Reproducibility of neurological examination, current perception and vibratory perception threshold.**

		H (n = 15)	DN- (n = 15)	DN+ (n = 14)		D20 (n = 19)
CURRENT PERCEPTION THRESHOLD						
					2 invest[1]	
Median nerve	2000 Hz	21.9	7.3	22.1	43.0	
	250 Hz	25.0	20.0	22.2	60.3	
	5 Hz	22.1	14.9	26.9	51.0	
					2 invest[1]	
Peroneal nerve	2000 Hz	13.3	20.2	13.8	13.3	16.0
	250 Hz	10.5	23.6	6.8	37.9	30.0
	5 Hz	14.0	29.3	62.0*	61.1	24.7
VIBRATION PERCEPT. THRESHOLD						46.8

* *Very large distribution in one individual; if this case was omitted, reproducibility fell to 17 %.*

[1] *These figures relate to a subgroup of 12 individuals (DN+) who were measured by two different investigators (inter-individual variation). All other values are from single investigators (intra-individual variation).*

Compare with reproducibility of neurological examination of 26.8% between two observers obtained in 12 patients of group 3 (see text).

in patients with neuropathic disturbances. Particularly the inter-observer variability of CPT at 5 and 250 Hz is a matter of concern.

Reproducibility of VPT testing in our hands is poor. More favourable figures have been reported [11,16], but also large differences, especially in patients with more neurological disturbances are also cited [30]. Perhaps unilateral measurements accounts partly for the high variation [31]. In our protocol, VPT's were obtained at the end of a session, so participants could also have been less concentrated because of fatigue.

In conclusion, the quantitative sensory threshold measurements used in this study are only of limited value, mainly because of high variability and poor reproducibility. CPT testing at 2000 Hz is a good reproducible but rather insensitive method of measurement. We have not been able to identify a correlation between the different electric sine wave frequencies tested and tests of large and small fibre function. VPT testing in our hands and in the groups we tested was poorly reproducible.

To detect and characterize diabetic polyneuropathy, a careful detailed clinical examination seems best suited and fairly reproducible. When additional sensory perception testing is to be performed, measurements should be repeated and preferably performed by a single investigator. CPT testing at 2000 Hz offers a useful tool, but its advantages over existing devices are probably limited.

A strong appeal should be made to develop uniform, internationally accepted, neurologic examination scores. Recent recommendations could be helpful in this regard [17].

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Chapter 3

An automated computerized method using Finapres for measuring cardiovascular reflexes.

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Abstract.

The major drawback of the cardiovascular reflex tests used to study autonomic failure is the time involved in calculating the results. To overcome this disadvantage, we have developed an automated computerized program using a FINger Arterial PRESsure instrument for the measurement of beat-to-beat heart rate and blood pressure on a finger. This program calculates heart rate variability during three standardized tests, forced breathing, standing up and the Valsalva manoeuvres, and records blood pressure values in response to standing up and sustained handgrip. The time taken to perform the test and to calculate the results is usually 25 min.

The reproducibility of the tests in 21 normals was comparable to the reproducibility obtained with conventional test methods using an ECG and sphygmomanometer.

In addition, we determined the age-dependent normal values of the seven test parameters in 124 subjects aged 20 to 90 years.

Using this program in 10 patients with long-standing (14-50 years) complicated diabetes, in each case of them four or more abnormal tests results were found.

Introduction.

Cardiovascular reflex tests based on heart rate and blood pressure variability during forced breathing, standing up, the Valsalva manoeuvre and sustained handgrip are most commonly used to detect diabetic autonomic neuropathy [1,2]. Because much time is required to elaborate the test results, automated programs have been developed so far on limited scale, using heart rate variability recorded with an ECG [3,4]. Using a Finapres (from FINger Arterial PRESsure) instrument, heart rate (beats/minute) and blood pressure (mmHg) can continuously be registered from the finger non-invasively. The Finapres is based on servoplethysmomanometry, employing the volume clamp technique [5-7]. The total finger arterial volume under an unloading finger cuff is measured with an infrared plethysmograph and despite changes in intra-arterial pressure this volume is clamped by modulating cuff pressure in parallel with intra-arterial pressure using a wide bandwidth electro-pneumatic servo-system. During actions such as the Valsalva manoeuvre, sustained handgrip and postural changes, the brachial to finger pressure differs only quantitatively, but the pattern of blood pressure changes is similar [8,9].
By connecting the Finapres to a personal computer, the opportunity exists to develop an automated program for calculation of the test results within a short period of time.

Methods.

The Finapres (Finapres, model 5 TNO; Amsterdam, The Netherlands) was connected with an IBM compatible computer using normal co-axial or shielded cable with a standard BNC connector. In Turbopascal 3.0 (Borland International Inc; Scotts Valley, USA) a program was written based on a timeclock. Before each test the clock was started and the recorded digital signal of the Finapres was used to calculate the programmed test results. The manoeuvres were performed after rest and in the posture described by Wieling and co-workers [10,11]. Before the start of the test, the subjects were trained to perform the manoeuvres correctly. The Finapres cuff was wrapped around the middle finger of the non-dominant arm, which was fixed at heart level.

Control systolic blood pressure, diastolic blood pressure, mean blood pressure and heart rate were calculated as the mean values during 30 s before the onset of each manoeuvre.

Forced breathing.

The subjects performed six consecutive maximal respirations in the supine position at a rate of six breaths/min after 5 min of supine rest. Within 1 s of the start of inspiration the heart rate rises quickly to a peak at about 3 s before maximal inspiration and a minimal heart rate is reached about 6 s after the beginning of expiration (Fig. 1).

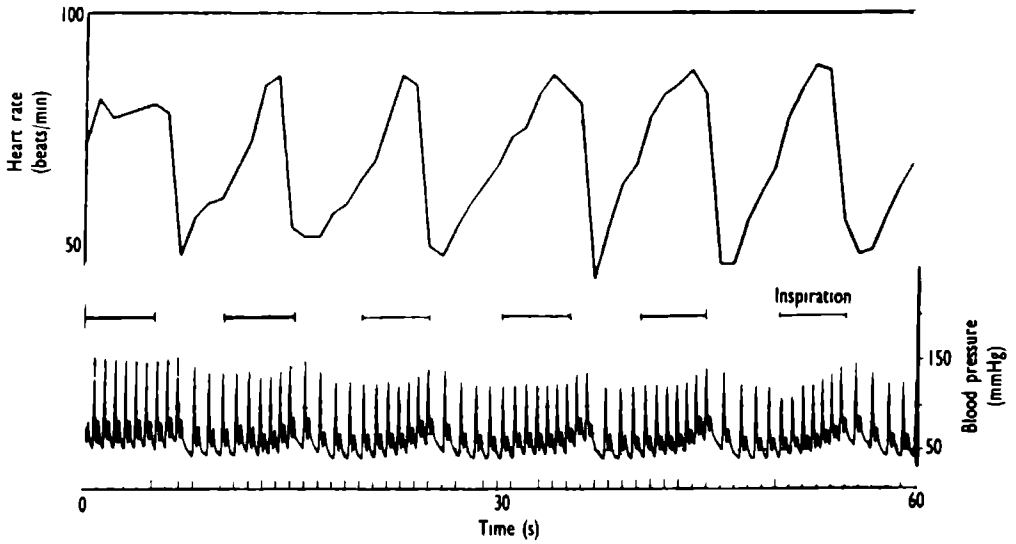

Fig 1 **Finapres recording during forced breathing.** The systolic and diastolic blood pressure are measured in mmHg The heart rate is computed by the Finapres instrument by measuring the time between two upstrokes The graph of the heart rate variability recorded by the personal computer is shown as the upper part of the figure The mean difference between the highest and lowest heart rate during each of the six breathings is calculated (*I-E difference*)

During this test the subjects synchronized inspiration and expiration with the program clock on the computer monitor. The difference between maximal and minimal heart rate thereafter (inspiration-expiration difference, *I-E difference*) during each 10 s cycle was measured and averaged for six cycles [1,12,13].

Standing up manoeuvre.

Standing up was started on verbal command, after 5 min of supine rest. The manoeuvre was performed in 2-3 s and the subjects remained in the upright position for 2 min.

The blood pressure response to standing is shown in the lower part of Fig. 2. After an initial jump following the performance of the manoeuvre, the blood pressure decreases to a minimum at about 8 - 10 s after standing up.

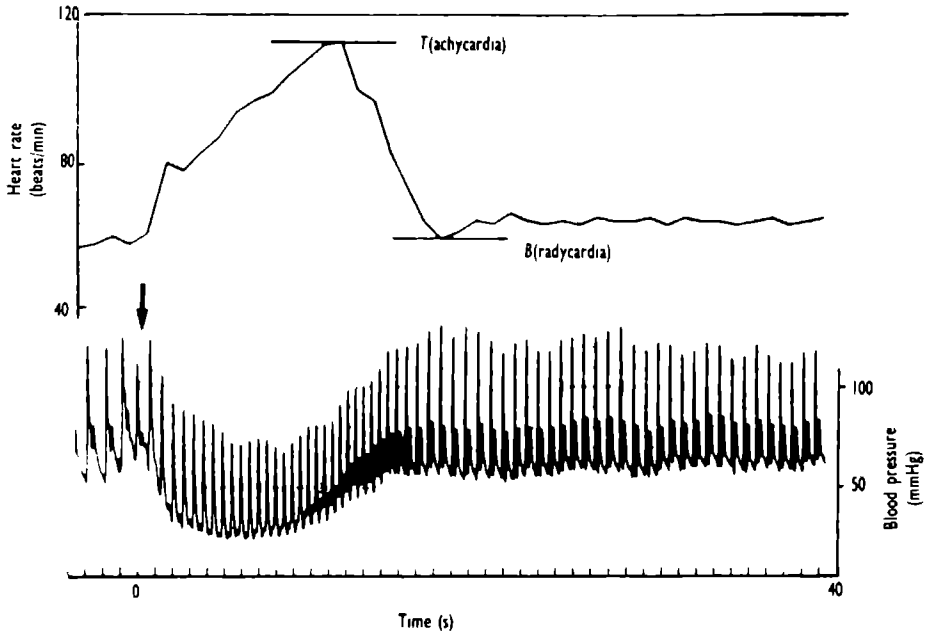

Fig. 2. **Finapres recording of a standing up manoeuvre and graph of the variation in heart rate recorded by the personal computer during standing up.** The highest heart rate after standing up divided by the lowest thereafter is the *T/B ratio*. The arrow indicates the beginning of the standing up manoeuvre.

Thereafter a blood pressure overshoot is seen [14]. The program calculated the difference between averaged systolic (*Δ BPsys.*) and diastolic (*Δ BPdias.*) blood pressure between 50 and 80 s after standing up and during the control period.

Standing up evoked an immediate sharp increase in heart rate to a peak occurring at about 15 - 20 s. Thereafter heart rate decreased rapidly to the control level [15] (Fig. 2, upper part).

The difference between maximum heart rate after standing and control heart rate (*ΔHR-max*) and the quotient of maximum heart rate and minimum heart rate after standing up (tachycardia/bradycardia ratio, *T/B ratio*) were calculated [16,17,].

Valsalva manoeuvre.

The subjects were instructed to maintain an expiratory pressure of 40 mmHg during 15 s, by means of forced expiration into a mouthpiece connected to a sphygmomanometer. Closure of the glottis was prevented by a small leak to maintain a flow of air. The manoeuvre was performed three times in the sitting position, each after 1 min rest.

The cardiovascular responses to a Valsalva manoeuvre can be divided into four phases [18] (Fig. 3). Immediately after the onset of the manoeuvre, a brisk rise of systolic and diastolic arterial pressure and a reduction in heart rate are seen (phase I). After 4 s a fall and subsequent partial recovery of arterial pressure and an increase in heart rate characterize phase II. Immediately after the release of straining a sudden, brief (1-2 s) further reduction of arterial pressure and an elevation of heart rate occur (phase III). In phase IV, an elevation of systolic and diastolic arterial pressure above control level (overshoot) is seen, accompanied by a bradycardia relative to the control heart rate.

Although the cardiovascular responses to a Valsalva manoeuvre are complex, the computer program calculated only the *Valsalva ratio*, defined by the maximum heart rate during the manoeuvre divided by the minimum heart rate after the manoeuvre [19,20].

Sustained handgrip.

Three minutes of squeezing a calibrated handgrip dynamometer at 30% of maximal voluntary contraction in the sitting position was performed, 2 min after the last Valsalva manoeuvre. During this isometric exercise, heart rate, systolic blood pressure and dias-

tolic blood pressure increase. The difference between the average maximum diastolic blood pressure over 5 s during the handgrip and baseline value (*delta BPdias.*) was calculated [21].

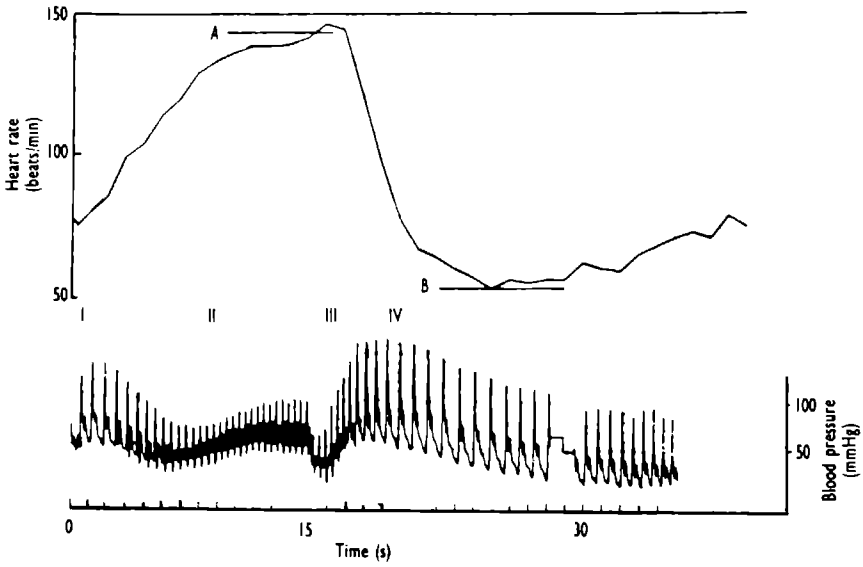

Fig. 3. **Finapres recording and graph of the heart rate variability recorded by the personal computer during the Valsalva manoeuvre.** The highest heart rate during the manoeuvre (A) divided by the lowest heart rate after the manoeuvre (B) is the *Valsalva ratio* (I,II,III and IV indicates the four different phases, see the text).

Statistical analysis

Statistical analysis was performed using the SAS software package (Statistical Analysis System, Cary, North Carolina, USA). Methods included a two sampled *t*-test to determine difference in test results between men and women. Calculation of a Pearson correlation coefficient was used to assess the relationship between parameters of autonomic

function and age. For each test parameter, a mean and 5th and 95th percentiles were calculated.

The reproducibility of repeated tests was determined as the coefficient of variation; CV = 100% x SESO/ [½ x (mean first test + mean second test)]. SESO is the standard error of a single observation and is calculated by the formula:

$$\text{SESO} = \sqrt{\sum_{i=1}^{n} \frac{(X1_i - X2_i)^2}{2n}}$$

in which $X1_i$ - $X2_i$ is the difference between the first and the second test X in subject i and n the number of paired observations.

Results

Reproducibility

In 21 healthy subjects (age 22 - 32 years) the tests were performed on two different occasions, under standardized conditions in a climate room (mean temperature 24.3 ± 0.3 ^{0}C). The subjects had normal blood pressure (< 130/80 mmHg), regular heart rate and were without medication. Each subject was tested on the same time of the day. All the subjects refrained from caffeine- or alcohol-containing beverages for 12 h before the test, and the three smokers also refrained from smoking on the day of the test.

The SESO and CV of the control blood pressure and heart rate before each manoeuvre were respectively between 2.6 and 7.0% and 4.4 and 9.1%. In eight normal subjects the tests were performed with the conventional method, using an ECG and a sphygmomanometer. The SESO and CV of the test parameters in these two groups are shown in Table 1. With the exception of the $\Delta BPsys.$ during the standing up test, reproducibility was moderate to good. Although the blood pressure parameters were slightly more reproducible with the automated program, no significant differences were noted.

Table 1. **Reproducibility of the 7 test parameters.** *A* = 21 normal subjects tested with the automated program. *B* = 8 normal subjects tests performed with ECG and sphygmomanometer. SESO = standard error of a single observation, CV = coefficient of variation.

Test and parameter(s)	SESO		CV (%)	
	A	*B*	*A*	*B*
Forced breathing				
I-E difference	3.3	2.6	11.4	11.1
Standing up				
Δ HRmax	4.6	3.8	11.3	9.3
T/B ratio	0.1	0.1	9.2	8.6
Δ BPsys	7.3	5.1	162.2	204.3
Δ BPdias	3.3	8.3	29.2	65.6
Valsalva manoeuvre				
Highest ratio	0.2	0.2	9.4	8.6
Sustained handgrip				
delta BPdias	5.8	9.1	18.6	36.9

Normal values.

A total of 124 healthy normal subjects (66 males and 58 females) were studied. The subjects were reasonably distributed according to age and sex, except in the age category over 80 years there were only three men. Diabetic patients were excluded, as where those with cardiovascular disease and those taking drugs known to affect heart rate or blood pressure. The blood pressure was normal according to WHO criteria and all subjects had a regular pulse. None of the subjects complained of a Raynaud's phenomenon or had other abnormalities of the hand and fingers.

Forced breathing. The mean *I-E difference* was age-dependent. Fig. 4a shows the relation between age and mean *I-E difference* with 5th and 95th percentiles.

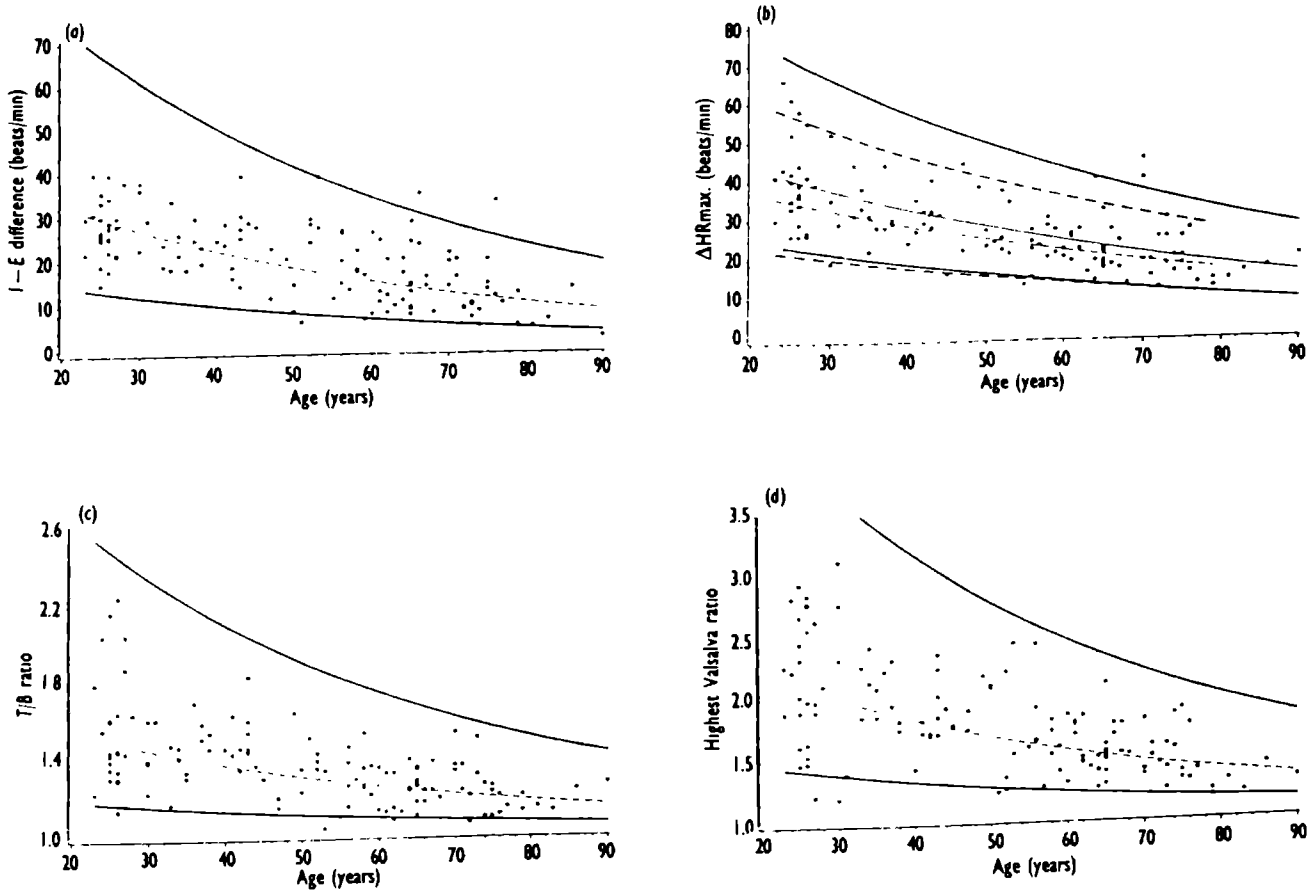

Fig. 4. **Age related decline in (a) *I- difference*, (b) Δ *HRmax*, (c) *T/B ratio* and (d) highest of three *Valsalva ratios* in normal subjects.** In the figure the mean and the 5th and 95th percentiles about the line of best fit are shown. In (b) ——— indicates men and - - - - indicates women.

Standing up. Heart rate variability during standing up was age-dependent. A sex difference was found for *Δ HRmax.* ($p < 0.02$, Fig. 4b.), but not for the *T/B ratio* (Fig. 4c). The 5th percentiles of the *ΔBPsys.* and *ΔBPdias.* were respectively -3.2 and 0.7 mmHg.

Valsalva manoeuvre. Fig. 4d shows the mean with 5th and 95th percentile of the *highest Valsalva ratio* of the three performed Valsalva manoeuvres. The ratio appeared to be age-dependent.

Sustained handgrip. *Delta BPsys.* ($p < 0.008$) and *delta BPdias.* ($p < 0.0001$) were sex-dependent, but age did not influence the test results. The 5th percentile of the *delta BPdias.* for men was 7.8 mmHg and for women 6.8 mmHg.

Diabetic patients.

Using this program 10 patients with long-standing (14 - 34 years) complicated diabetes were studied. Three patients (two females aged 29 and 30 years and a male aged 50 years) complained of orthostatic hypotension. The other patients had severe diabetic neuropathy of the legs, no vibration sense and absent knee and ankle yerks. Each patient had more then four abnormal results (below 5th percentile corrected for age and gender, Table 2). The heart rate parameters were more often abnormal than the results of tests based on the variability in blood pressure. In this group of diabetic patients the *I-E difference, Δ HRmax, T/B ratio* and the *Valsalva ratio* were always below the 5th percentiles of normal.

Discussion.

We developed an automated program to detect diabetic autonomic neuropathy, using a personal computer and a Finapres to record blood pressure and heart rate variability during standard cardiovascular reflex tests. Within 25 min the tests can be performed and the results calculated.

Table 2. **Test results in 10 patients with long-standing complicated diabetes.**

Age en sex (yrs)	♀29	♀30	♂35	♀37	♂38	♂42	♂46	♂50	♂58	♀64
Duration of DM (yrs)	16	17	31	27	24	14	25	17	25	34
I-E difference (beats/min)	4.3*	4.5*	5.8*	4.2*	2.7*	2.5*	3.7*	3.3*	1.6*	3.3*
Δ HRmax (beats/min)	15*	10*	7*	13*	4*	6*	5*	5*	0*	6*
T/B ratio	1.02*	1.00*	1.11*	1.03*	1.02*	1.05*	1.06*	1.06*	1.00*	1.03*
Δ BPsys (mmHg)	-65*	-37*	27	-6*	6	27	4	-63*	-8*	24
Δ BPdias (mmHg)	-31*	-20*	19	0*	14	19	0*	-12*	-5*	11
Valsalva ratio	1.06*	1.06*	1.20*	1.00*	1.12*	1.04*	1.06*	1.05*	1.05*	1.03*
delta BPdias (mmHg)	3*	-5*	14	27	11	7*	22	-9*	32	16

** Below 5th percentiles of normal*

The blood pressure profile is printed by the Finapres at the same time. This makes it possible for the investigator to detect irregularities caused by time delay between starting the computer and initiating the manoeuvres, by extrasystoles or by artefacts.

In 14 of the 124 subjects, a part of the program had to be repeated (forced breathing was repeated six times because of coughing). In 28 of subjects tested it was necessary to correct the test results afterwards using the saved data. The most frequent reason for correction was the presence of premature beats in the elderly.

The reproducibility of the heart rate variability test parameters was comparable with results reported in literature [8,22-24], whereas reproducibility of the blood pressure variability was slightly better than with the conventional method. The beat-by-beat blood pressure monitoring by the Finapres allows the possibility of analysing in details the whole blood pressure curve after a given stimulus. Calculating the mean over 30 s (standing up) or a more precise detection of peak blood flow pressure changes (sustained handgrip) explains the slightly better reproducibility compared to the traditional sphygmomanometer approach.

The age-dependent 5th percentiles of the heart rate parameters was also found by others using different methods for measuring heart rate and blood pressure [23-27]. However, the relation of Δ *HRmax.* and sex was in contradiction with literature [23,25,27]. The sex-related 5th percentile of *delta BPdias.* during handgrip was also found by Gautschy et al, although in their study the difference did not reach statistical significance [27].

The seven test parameters used are generally recommended for detecting diabetic autonomic neuropathy; moreover the program calculates also automatically the difference between the highest heart rate during and the lowest heart rate after the Valsalva manoeuvre [23,28], the blood pressure overshoot [29] after the Valsalva manoeuvre and delta systolic blood pressure and heart rate increase during handgrip [21,30]. A number of less frequently used parameters can easily be programmed, for instance expiration/inspiration ratio [12] and heart rate variation [31] during forced breathing or 30:15 ratio after standing up [32]. As all data are saved on the disc, future calculation can be carried out at later convenience.

With this automated program, diabetic patients can easily be tested by a trained nurse for dysfunction of the autonomic nervous system. It creates the opportunity to study a

large number of patients in a standardized way during a clinical trial or for screening diabetic patients before surgery [33].

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Chapter 4

Evaluation of two sympathetic cutaneous vasomotor reflexes using laser Doppler fluxmetry

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Submitted

Abstract

Disturbances in sympathetic cutaneous vasomotor reflexes may be of pathogenetic importance in several microvascular problems, as for example the neuropathic foot in patients with diabetes.

Laser Doppler fluxmetry (LDF) enables to study the influence of sympathetic reflexes on skin blood flow. A matter of concern is the high variability of skin blood flow and its reactivity to sympathetic reflex tests resulting in a poor reproducibility.

In this study we evaluated two sympathetic stimulation tests; distant cooling and inspiratory gasp, and their influence on LDF measured skin blood flow of the big toe in 63 healthy volunteers.

No age- or sex-dependency of the LDF test results were found. Absolute and relative LDF decrease during distant cooling was highly variable between the subjects (percentage LDF decrease: mean $\pm$ SD; 0.7 $\pm$ 5.3%), compared to a percentage LDF decrease of 46.5 $\pm$ 3.1 % during an inspiratory gasp test. The reproducibility however was better for the distant cooling test (coefficient of variation [CV]: distant cooling; 5.8%, inspiratory gasp test; 35.4%). By the use of a thermostatically controlled LDF probe holder fixed at a temperature of 36°C, the short-term reproducibility of the two sympathetic vasomotor test did not improve, probably because of a steady increase in baseline skin blood flow during the test. Surprisingly long-term variability of the percentage LDF decrease during the inspiratory gasp test, performed with the heated LDF probe was lower, compared to the short-term variability (CV 19.2% versus 39.0%, $p < 0.05$).

In conclusion; to study sympathetic skin vasomotor reflexes with LDF, vasoconstriction during inspiratory gasp test was more uniform, compared to distant cooling test. Measuring skin blood flow reactivity with a heated LDF probe (36°C) did not improve reproducibility.

Introduction

Sympathetic dysregulation of skin microcirculation is supposed to play a pathogenic role in several syndromes like Raynaud's phenomenon [1], post-traumatic reflex dystrophy of the extremities [2] and diabetic neuropathy [3]. In diabetic patients sympathetic peripheral autonomic neuropathy results in remarkable arteriovenous shunting in the foot [4]. Despite an increased peripheral skin blood flow ulcerations may easily develop, with major healing problems.

Often cardiovascular autonomic reflex tests are used to determine autonomic neuropathy [5]. Although these cardiovascular reflex tests have increased our understanding of autonomic dysfunction in diabetes, they do not provide a sensitive index of sympathetic dysfunction in the skin microcirculation of the foot [6].

To assess focal autonomic nervous dysregulation of the skin blood flow, laser Doppler fluxmetry (LDF) can be used, to measure variations in flow induced by sympathetic stimulation. LDF enables to measure blood flow of the superficial skin microcirculation continuously and non-invasively [7,8]. The laser light usually penetrates to a depth of less than 1.5 mm [9], corresponding with the location of the nutritive capillaries and at least the superficial part of the arteriovenous shunt system [10]. The latter are controlled by dense sympathetic nerve endings, and are responsible for the thermoregulatory adjustment of skin blood flow to environmental conditions.

Therefore sympathetic skin microcirculatory vasomotor responses can be quantified by LDF measurements during for example cold challenge [11,12,13]. Another manoeuvre to stimulate sympathetic skin fibers is an inspiratory gasp [12,13,14]. The major problem in clinical studies with these LDF sympathetic vasomotor tests is the variability and possible age-dependency [12,15,16].

In this study short-term reproducibility and age-dependency in 63 healthy volunteers were analyzed. Furthermore short- and long-term reproducibility were studied. To standardize local skin temperature, a thermostatically controlled LDF holder set at 36^0C was used in an attempt to minimize the variability of the vasoconstrictor response.

Methods

Study 1.

Sixty nine healthy volunteers (age: 20 to 70 years; 34 women) were selected after a news paper announcement. All gave informed consent to a protocol approved by the local ethics committee. Exclusion criteria were: blood pressure above 140/90 mmHg; an ankle/brachial index below 1.0 and above 1.3; random blood glucose concentration above 8.0 mmol/l, HbA1c value above 6.3 %, a total cholesterol above 6.5 mmol/l, smoking or the use of medications known to influence skin blood flow, or an abnormal ECG. To exclude autonomic dysfunction, five cardiovascular reflex tests were performed with an automated program using a Finapres device [5]. If more than one test result was underneath the 5^{th} percentile the person was excluded. Sixty three subjects met these criteria and were included.

The microcirculation measurements were conducted in a temperature controlled laboratory (24.1 $\pm$ 0.4°C (mean $\pm$ SD); relative humidity 60.5 $\pm$ 3.8%), after abstinence of alcohol or caffeine containing beverages for 12 h and a meal for 2 h preceding the tests. LDF was measured with a Periflux Pf1d (Perimed, Linköping, Sweden) in Perfusion Units (PU) [16]. The Periflux was adjusted to an upper frequency limit of 12 Hz and the output circuit time of 3 s and gain of 3.

After 30 min acclimatization in the supine position the laser Doppler probe was attached to the plantar side of the left big toe, by means of a double sided adhesive tape. A toe cuff was wrapped around the base of the left big toe. Skin temperature in °C was measured using a thermocouple (Ellab instruments, Copenhagen, Denmark) that was attached near the LDF probe.

After 15 min baseline registrations skin blood flow was arrested during 5 min by inflating a toe cuff to suprasystolic pressure, followed by a sudden deflation (Postocclusive Reactive Hyperaemia test, PRH). During the last minute of the circulation arrest a biological zero was determined and subtracted from all the LDF measurements [17]. Thirty minutes later the right hand was immersed in a water bath of 10°C during 1 min. Baseline LDF during the last minute before the manoeuvre and LDF during distant cooling were determined. Absolute and percentage decrease in LDF was calculated. The first

inspiratory gasp test was performed 15 min later. The subjects were asked to take a deep breath as quick as possible and to hold it for 10 s. Baseline LDF during the last minute before the gasp and the lowest LDF during the gasp were calculated, to obtain the absolute and relative LDF decrease. After 5 min baseline registration a second inspiratory gasp was performed and 15 min later a second distant cooling test.

Study 2.

In an attempt to improve reproducibility, the sympathetic skin vasomotor reflexes were performed in 10 volunteers by using a heated (36^0) LDF probe holder, placed on the plantar side of the left big toe. Distant cooling and inspiratory gasp test were performed in duplicate, after 10 min baseline registration, in the same way as described above, including a PRH test. On a second occasion 2 weeks later all tests were repeated with the same heated probe holder. LDF parameters were calculated as mentioned before. Furthermore the LDF test parameters were estimated by two independent investigators to analyze interobserver variability.

Statistical analysis.

The data are expressed as mean and standard error (SE), unless stated otherwise. The coefficient of variation (CV) of the test results was calculated by the formula:

$$\text{CV-S} = \sum \frac{\text{SD (1a and 1b)}}{\text{mean (1a and 1b)}} \times 100\%;$$

$$\text{CV-L} = \sum \frac{\text{SD (1a and 2)}}{\text{mean (1a and 2)}} \times 100\%;$$

on which CV-S represents short-term reproducibility, CV-L long-term reproducibility. The symbols 1a and 1b refer to the results of the tests performed on the same day and the symbol 2 refers to the results from the separate occasion. In the same way inter-observer reproducibility was calculated, by comparing the LDF parameters estimated by the two investigators.

Wilcoxon signed rank test is used to study sex-differences, and a Spearman correlation coefficient to analyze age-dependency. A p-value < 0.05, two-sided, was regarded statistically significant.

Results

Study 1.

The subjects were reasonably distributed according to age and sex, except in the age category 40 to 60 years where there was a female predominance.

Only baseline laser Doppler flux before the first inspiratory gasp test was significantly correlated with age ($r = 0.26$; $p < 0.05$), while none of the other test parameter was age-dependent.

No significant differences in any of the parameters between sexes were found. Baseline LDF before each test was correlated with the skin temperature ($r = 0.73$ - 0.81; $p < 0.0001$). None of the other LDF parameters was significantly correlated with temperature.

Between the subjects, absolute and percentage LDF decrease during the distant cooling test were highly variable. Some showed an increase in LDF during cold challenge (Fig. 1).

During inspiratory gasp only twice an increase in LDF (17% and 18%) and 7 times no changes in LDF were seen. Short-term reproducibility was better for the distant cooling test, compared to the inspiratory gasp test (Table 1).

Study 2.

In 10 healthy volunteers (5 women, age 26.3 ± 4.8, [mean $\pm$ SD]) short- and long-term

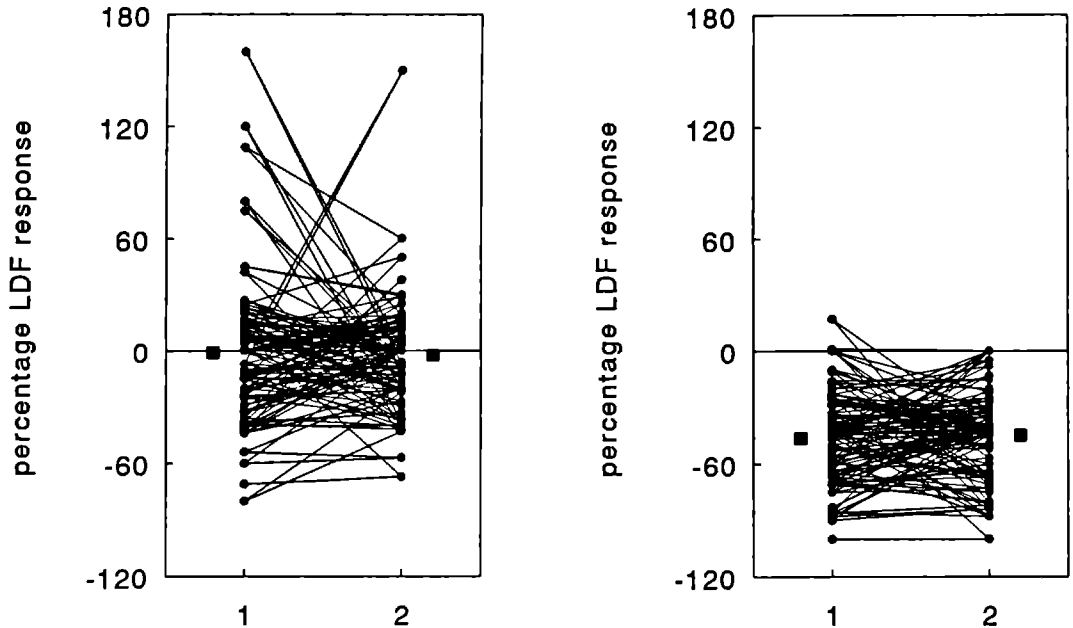

Fig. 1. **Individual test results during study 1 of the distant cooling test** (left part) **and inspiratory gasp** (right part). Means are indicated by black squares. reproducibility (CV-S = 39.0%, Table 2).

reproducibility were calculated. Surprisingly long-term reproducibility (CV-L = 19.2%) of the percentage LDF decrease was better ($p < 0.05$) compared to the short-term reproducibility (CV-S = 39 0%, Table 2). In all subjects a decrease of LDF was seen during distant cooling and only one subject showed no decrease during the third inspiratory gasp test.

Interobserver reproducibility of the LDF estimations was good (CV between 1.7% for baseline LDF and 6.8% for percentage LDF decrease during the inspiratory gasp test).

Table 1 **Test results and short-term reproducibility of the two sympathetic cutaneous vasomotor reflex test using laser Doppler fluxmetry in 63 healthy volunteers.** Mean ± SE and short-term coefficient of variation (CV-S)

	Mean		CV-S (%)
	Test 1a	Test 1b	
DISTANT COOLING			
Baseline LDF	18 8 ± 1 7	16 9 ± 1 5	17 0
Absolute LDF decrease	1 3 ± 0 8	0 7 ± 0 5	11 8*
Percentage LDF decrease	0 7 ± 5 3	2 5 ± 3 9	5 8**
INSPIRATORY GASP			
Baseline LDF	16 5 ± 1 4	16 6 ± 1 5	13 7
Absolute LDF decrease	7 7 ± 0 8	7 6 ± 0 8	40 7*
Percentage LDF decrease	46 5 ± 3 1	44 9 ± 3 0	35 4**

Significant difference ($p < 0.05$) between the CV-S of the absolute LDF decrease (*) and percentage LDF decrease (**) of both tests

Discussion

In this study two sympathetic skin vasomotor reflexes of the toe using LDF were evaluated No age-dependency or sex-dependency of the LDF parameters were found There was an enormous variability of the LDF responses between the subjects, especially during distant cooling However reproducibility was better compared to the inspiratory gasp test The short-term reproducibility of LDF parameters of the two skin vasomotor tests did not improve by using a thermostatically controlled LDF holder at 36^{0}C and long-term reproducibility was even better

The great variability in the sympathetic vasomotor reflexes is understandable The two sympathetic stimulation tests used in this study have different afferent pathways somatic afferents with the distant cooling test [10] and chest wall and lung inflation receptors [18] with the inspiratory gasp However both share the same efferent pathway Differences in afferent pathways may influence sympathetic regulated skin vasomotor reflex, measured with LDF During the tests, skin sympathetic fibres are responsive to thermal and emotional stimulation as well [19,20] Furthermore skin blood flow measured with LDF may also be modulated by other mechanisms, including local autoregulatory mechanisms [21], veno-arteriolar reflexes [22], circulating metabolites and humoral factors [23], especially endothelial secretory products [24], hormonal status [25] and blood rheology [26] Therefore spontaneous fluctuation in skin blood flow may not depend exclusively on oscillations in the activity of the sympathetic microvascular innervation [27]

Skin blood flow responses to various types of stimuli seems profoundly influenced by the thermoregulatory state of the subject [28] The maintenance of a stable skin temperature of 34 - 35^{0}C is important in the LDF response during sympathetic stimulation [12] At lower temperatures vasoconstriction occurs, while at higher temperature vasoconstrictor reflexes are impaired By increasing skin temperature variability of the vasoconstrictor response on sympathetic stimulation is possibly minimized [28] Kahn and colleagues assessed skin blood flow with LDF of the left index finger, after placing the arm in a water bath maintained at 43^{0}C, inducing a higher and relatively stable fingertip blood flow [29] Reproducibility of an inspiratory gasp test and distant cooling

Table 2 **Test results and reproducibility in 10 healthy volunteers of the two sympathetic skin vasomotor reflexes using a thermostatically controlled holder of the LDF probe at 36°C.** Mean ± SD and short-term coefficient of variation (CV-S) and long-term coefficient of variation (CV-L)

	Test 1a	Test 1b	Test 2	CV-S (%)	CV-L (%)
DISTANT COOLING					
Baseline LDF (PU)	44 6 ± 18 4	49 9 ± 17 0	46 5 ± 20 0	22 5	8 2
Absolute LDF decrease (PU)	11 3 ± 8 0	9 0 ± 7 7	12 5 ± 5 5	29 8	23 7
Percentage LDF decrease (%)	29 8 ± 21 0	22 3 ± 20 1	32 6 ± 18 9	40 8*	19 9*
INSPIRATORY GASP					
Baseline LDF (PU)	47 0 ± 18 6	50 2 ± 16 8	46 4 ± 18 0	18 9	3 3
Absolute LDF decrease (PU)	13 9 ± 7 4	13 4 ± 6 9	13 0 ± 7 5	30 4	20 1
Percentage LDF decrease (%)	31 8 ± 14 8	25 8 ± 14 8	28 6 ± 13 7	39 0**	19 2**

Significant difference between CV-S and CV-L of the percentage LDF decrease of the distant cooling test (*) and inspiratory gasp test (**)

test performed in this way seems better. Yet the responses in hands and feet differ [27]. In contrast to the present study baseline LDF was determined over a longer period of 5 min versus 1 min, and as LDF response during distant cooling the lowest LDF value was taken. Because of both the variability in baseline LDF and in the absence of a clear LDF decrease this way of calculating the test result seems not to be justified.

In the present study local skin temperature was standardized with a thermostatically controlled LDF probe at 36^{0}C. Baseline LDF increased and a more uniform LDF response during especially the distant cooling test was seen. Despite short-term producibility did not improve, probably explained by changes in baseline condition during local heating, resulting in variations in vascular sympathetic response. In agreement with this possibility is the lower long-term variability, because the time passed between starting the LDF recording with the heated probe and both test 1a and 2 were the same.

An alternative to improve reproducibility of the inspiratory gasp tests, is standardization of the respiratory manoeuvre with a spirometer. This should be evaluated in future studies.

We found no age-dependency of the skin vasomotor reflexes, as reported by Kahn and colleagues [29]. This is possibly explained by a spatial organization of vasomotor control, because they performed LDF measurements on the finger instead on the toe [27].

In conclusion sympathetic skin vasomotor reflexes measured with LDF at the toe are highly variable. Of the two sympathetic stimulation tests analyzed in the present study, inspiratory gasp showed the most uniform response, although the reproducibility is still a matter of major concern.

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Chapter 5

Skin vasomotor reflexes during inspiratory gasp; standardization by spirometric control

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Submitted

Abstract

Vascular smooth muscle contraction within arteriovenous anastomoses (AVA) in the skin is mediated by sympathetic stimuli. The inspiratory gasp test (IG-test) triggers the sympathetic nervous system, resulting in a decrease in AVA skin blood flow, which is measured by laser Doppler fluxmetry (LDF). We studied the reproducibility of the IG-test under careful standardized respiratory conditions.

In 19 healthy volunteers with a mean skin temperature during the experiment above 28 degrees Celsius (= °C) 13 IG-tests were performed, either under spirometric control (n=6) or uncontrolled (n=4) and by using a negative pressure transducer (n=3). Starting the IG-test at end-expiration results in the largest volume of inspiration as expected, but starting at end-inspiration results in the most pronounced LDF-decrease ($p < 0.001$). Inspiration as fast as possible results in a more pronounced LDF-decrease, compared to inspiration in 5 s ($p < 0.02$). Continuously sucking negative mouth pressure results in a larger LDF-decrease in comparison with taking one deep breath and holding it for ten seconds ($p < 0.01$). However standardization of the IG-test does not improve reproducibility. An initially expanded position of the chest enhanced the response. Therefore, the involvement of stretch receptors in the lungs in the afferent pathway of this skin vasomotor reflex seems likely.

Introduction

Apart from arterioles and venules, superficial skin blood flow consists of two major systems: skin capillaries, serving nutritional demands, and the deeper localized arteriovenous anastomoses (AVA), with primarily a thermoregulatory function [1,2]. The AVA are most abundant in fingers, toes, ears and nose [1,3]. Under normal environmental conditions about 80-90% of acroteric skin blood flow results from AVA-flow [4].

Besides influences of myogenic [5], humoral [1] and metabolic factors [6] skin blood flow is primarily under local and systemic sympathetic alpha-adrenergic control [1,7]. Especially the AVA are richly supplied by sympathetic nerve endings, in contrast to the nutritive capillaries [1]. Cutaneous blood vessels do not receive cholinergic innervation [8]. Sympathetic stimulation is responsible for the contraction of the vascular smooth muscle within the vessel wall of the AVA resulting in a decrease of skin blood flow [9].

Sympathetic dysregulation of skin microcirculation is supposed to play a pathogenic role in several diseases and syndromes. In diabetic patients [10,11,12,13] sympathetic peripheral autonomic neuropathy results in remarkable arteriovenous shunting in the neuropathic foot. In patients with Raynaud's phenomenon AVA vasoconstriction due to sympathetic nervous system hyperactivity is hypothesized [14].

Focal abnormalities in sympathetic function are not always apparent from global assessments by cardiovascular autonomic function tests. Moreover skin sympathetic dysfunction may occur in the absence of clinically detectable abnormalities of cardiovascular autonomic function [15,16].

In a previous study two sympathetic skin vasomotor reflexes, to assess focal autonomic nerve damage were analyzed [17]. Skin blood flow was measured by laser Doppler fluxmetry (LDF) [17,18]. Distant cooling resulted in an enormous variation in LDF change between the subjects. A decrease as well as an increase was seen, making this test unsuitable for clinical studies. The inspiratory gasp test (IG-test), i.e. taking a deep breath as quick as possible and holding it for 10 s, showed a more uniform LDF response. Still the reproducibility of the test is a major problem [17,18,19].

Aim of this study was to standardize the way in which the IG-test is performed in order to achieve better standardization and reproducibility of the skin vasomotor response. For

this purpose the IG-test was monitored with a spirometer, to determine the influence of the moment in the respiratory cycle the IG-test is started, as well as velocity and volume of inspiration. Also the effect of continuously sucking negative mouth pressure on the skin vasomotor reflex was tested instead of taking one deep breath and holding it. Moreover data concerning the nature of the afferent pathway of the reflex were collected.

Methods

Subjects

Twenty-two healthy volunteers (fifteen males, seven females) with a mean (± SD) age of 27.0 (± 4.3) years volunteered for the study. All gave informed consent to the test procedure which was approved by the local ethics committee.

Subjects were non-smokers, used no medication (except for oral contraceptives (n = 6) [20]) and were clinically free from respiratory and cardiovascular disease. Diabetes mellitus was in particular excluded by a negative urine test for glucose. Because elderly individuals may have a diminished or highly variable vasoconstrictor responses to the IG-test [21] subjects had to be under 40 years of age. During the experimental session mean skin temperature had to be above 28°C [22].

The volunteers had to refrain from alcohol for 24 h, from caffeine for 12 h and to fast for 2 h preceding the tests. All tests were performed in a quiet climate room with an ambient mean temperature of 24.4 (±0.2) °C and a relative humidity of 58.3 (± 2.2) %.

Subjects were asked to empty their bladder prior to the test procedure. After at least 20 min of acclimatization in a comfortable supine position in the climate room the experiments were started.

Instruments

Skin blood flow was continuously recorded using a laser Doppler fluxmeter (Pf-1d,

Perimed; Stockholm, Sweden) which was adjusted to an upper frequency limit of 12 kHz, a gain of 3 and an output circuit time constant of 0.2 s. The output signal was calibrated using Periflux motility standard PF100 and expressed in Perfusion Units. After electrical zero calibration the LDF-probe was attached by a double-sided adhesive ring to the distal volar surface of the first left toe. The toe was chosen because of its abundant AVA-density. The analogue output signal of the LDF was continuously recorded by one of the pens of a two-channel recorder (BD 9, Kipp & Zonen; Delft, The Netherlands).

After acclimatization a stable baseline value (flux zero) was registered during 5 min before the series of IG-tests was started. Finally a small cuff around the proximal first left toe was inflated up to a suprasystolic level of 300 mm Hg for 5 min. The resulting biological zero was subtracted from all previous LDF-values [23].

Skin temperature, measured by a thermocouple (Ellab instruments; Copenhagen, Denmark) was noted before the start of the registration of the flux zero and at the start of each IG-test.

The respiratory cycle, volume and velocity of inspiration were registered using a wet spirometer (Lode spirometer; Groningen, The Netherlands).

Negative mouth pressure, which is well correlated with intra-thoracic pressure [24], was measured using a negative pressure transducer (Validyne CD23-c; Northridge, USA). This was connected with a 60 ml-syringe with a mouthpiece. A needle (diameter 1.1 mm and length 4.0 cm) served as a leak so the volunteer had to keep on sucking through the mouth to maintain a stable negative mouth pressure. The signal was continuously recorded by the two-channel recorder.

Calibration of the pressure transducer and the connected ink-pen was performed before each test series. During the use of spirometer and pressure transducer a nose-clip was used to prevent sucking or leakage of air through the nose.

Inspiratory gasp tests

There was an interval of at least 2 min between each of the 13 IG-tests. All IG-tests were at least once practised before the start of the experiments.

The first and last two IG-tests (IG 1-2 and IG 12-13) were performed without special

equipment. The LDF-response was registered while the subject was asked to take a breath as quickly and deeply as possible and to hold it for ten seconds. These IG-tests are called 'uncontrolled'.

IG 3-8 were performed under spirometric control. With IG 3-5 the subject was asked the same as with the 'uncontrolled' IG-test but the start of inspiration was monitored at three different moments of the normal respiratory cycle: at the end of expiration (IG3), during inspiration (half-way, IG4) and at the end of inspiration (IG5). IG 6-8 all started end-expiratory but were performed with three different velocities of inspiration: as fast as possible (in practice within 2 s, IG6), in about 5 (IG7) and in about 10 s (IG8). The total time-period of inspiring and holding breath lasted up to ten seconds.

IG 9-11 were performed using the negative mouth pressure transducer. The subject was asked to put the mouthpiece in his mouth while holding his breath. About a second later the subject was instructed to inhale as fast and as deep as possible and to keep sucking for ten seconds.

Parameters

LDF values are expressed in Perfusion Units (PU; range 0-1000), and corrected for the biological zero. The LDF registration during the IG-test shows a rapid decrease to a certain minimum (LDFmin), returning quickly to the baseline LDF. A representative example of the LDF registration is shown in Fig. 1. Baseline LDF values were averaged during the last minute before start of the IG-test. Absolute and percentage decrease were calculated as well as the time that passed between starting the IG-test and the moment of the lowest LDF value (Tmin, in s).

From the spirometric registrations velocity of inspiration (l/min) and volume (l) were calculated. If the subject inspired with high velocity the tangent of the curve, while at lower velocities the slope of the line between beginning of the inspiration and the moment of maximum volume, was used to calculate velocity of inspiration. The highest velocity and largest volume inspired at the 3rd or 6th IG-test were used to calculate percentage velocities and volumes from the other IG-tests. From the recorded negative mouth pressure the maximum and mean were noted (in cm H_2O).

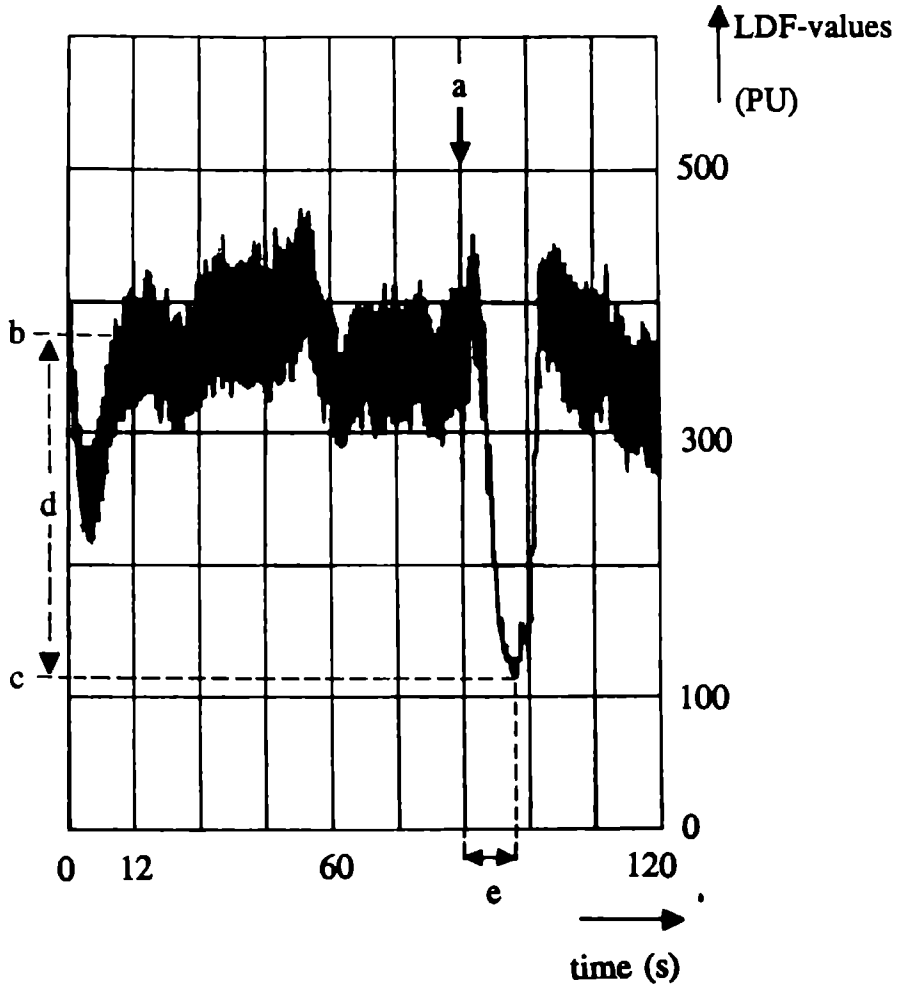

Fig. 1. **A representative example of the LDF registration during the inspiratory gasp test.**
a = start of the inspiratory gasp test; b = baseline LDF, mean of 1 min before start of the inspiratory gasp test; c = minimum LDF; d = absolute LDF-decrease; e = time to minimum LDF

Statistical analysis

All results are expressed as median because of a non Gaussian distribution of variables, unless stated otherwise. Minimum and maximum are indicated between brackets. LDF, spirometric and negative mouth pressure parameters are compared using the Wilcoxon's signed rank test; p-values of less than 0.05 (two sided) are considered significant.
Reproducibility is expressed as standard error of a single observation (SESO) and coefficient of variation (CV).
The SESO is calculated by the formula:

$$\text{SESO} = \sqrt{\frac{\sum_{i=1}^{n} (X1_i - X2_i)^2}{2n}}$$

in which d is the difference between the first and the second test in subject i and n the number of paired observations. The CV of two tests is calculated from the SESO by the formula:

CV = (SESO / mean of all subjects of the first test) x 100%.

Results

Three women were excluded from the study because of a mean skin temperature below 28°C. Two of them twice showed no decrease in LDF during the IG-test and one even once an increase in LDF. So the following results consider the remaining 19 subjects.

Influence of starting at different moments in the respiratory cycle (IG 3-5, Table 1)
As was expected starting the IG-test end-expiratory (IG3) results in the largest absolute volume of inspiration in comparison with starting during the inspiration (IG4) as well as end-inspiratory (IG5) (resp. $p < 0.01$ and $p < 0.001$). Comparing percentage volumes

of inspiration reveals the same (resp. $p < 0.01$ and $p < 0.05$).

Nevertheless, the IG-test starting at end-inspiration (IG5) results in the largest absolute LDF-decrease as compared to starting at end-expiration (IG3) and during inspiration (IG4) (IG3: 100 [40-260], IG4: 110 [50-350], IG5: 140 [70-490] PU; resp. $p < 0.001$ and $p < 0.01$). Percentage decrease in LDF shows the same comparing IG5 with IG3 ($p < 0.01$) (Table 1).

Table 1. **Absolute and percentage (median, [minimum - maximum] volumes of inspiration and absolute and percentage LDF-decreases at inspiratory gasp tests 3-8.**

Inspiratory Gasp (number)	Absolute volume of inspiration (l)	Percentage volume of inspiration (%)	Absolute LDF decrease (PU)	Percentage LDF decrease (%)
3	3.9 [2.3 - 4.7]	100 [81 - 100]	100 [40-260]	67 [21 - 87]
4	3.5 [2.3 - 4.2]	91 [74 - 100]	110 [50 -350]	66 [30 - 83]
5	3.4 [2.1 - 4.3]	89 [77 - 100]	140 [70 - 490]	73 [27 - 88]
6	3.8 [2.3 - 4.6]	100 [83 - 100]	150 [40 - 450]	70 [19 - 90]
7	4.1 [2.5 - 5.2]	100 [91 - 111]	120 [60 - 340]	60 [25 - 85]
8	3.9 [2.5 - 5.4]	100 [84 - 115]	130 [40 - 350]	62 [39 - 80]

The largest volume inspired, the IG-test starting at end-expiration and when inspiring as fast as possible (that means achieved at either IG3 or IG6), was considered the maximum volume of inspiration (100%). IG 3-5: IG-tests started at different moments during the respiratory cycle, resp. end-expiratory (IG3), half-way the inspiration (IG4) and end-inspiratory (IG5).
IG 6-8: IG-tests performed with different velocities of inspiration, resp. inspiration as fast as possible (IG6), in about 5 (IG7) and in about 10 s (IG8).

Influence of different velocities of inspiration (IG 6-8, Table 1)

Three different velocities were achieved Velocities of inspiration when inspiring in 5 and in 10 s are much lower compared to inspiring as fast as possible (IG6 240 [128-376], IG7. 41 [13-108] and IG8 23 [7-37] l/min , $p < 0.0001$)

Inspiration as fast as possible (IG6) results in a smaller volume of inspiration in comparison with inspiring in 5 s (IG7) ($p < 0.05$) This is also true for percentage volumes ($p < 0.05$)

In spite of the smaller volume of inspiration, inspiration as fast as possible (IG6) results in a larger absolute LDF-decrease compared to inspiring in 5 s (IG7, $p < 0.02$), and a larger percentage LDF-decrease compared to both inspiring in 5 and in 10 s (IG7 and IG8, resp $p < 0.02$ and $p < 0.05$)

When inspiring in 10 s the minimum LDF is reached significantly later in comparison with inspiring as fast as possible (Tmin resp 14 [3-19] and 12 [7-16] s, $p < 0.05$)

Sucking negative mouth pressure (IG 9-11, Table 1)

Continuously sucking negative mouth pressure results in a significantly larger absolute LDF-decrease in comparison with the 'uncontrolled' IG1, IG1 110 [30-270], IG9 140 [30-420], IG10 170 [30-350] and IG11 170 [30-420] PU, $p < 0.01$

Reproducibility (Table 2)

Between two 'uncontrolled' IG-tests the SESO concerning absolute LDF-decrease is about 3 PU and the CV about 24% When expressed in percentage LDF-decrease these figures are about 13 and 23 %

The SESO and CV are smaller between two 'uncontrolled' IG-tests performed in succession (IG1-IG2 and IG12-IG13) than between two IG-tests of which the second is performed after a lapse of time (IG1 and IG12)

The IG-tests 3 and 6 were performed starting at the same moment during the respiratory cycle, i c at the end of the expiration, and with highest velocity of inspiration

The SESO and CV between these two IG-tests (IG3 and IG6), is not better than between two 'uncontrolled' IG-tests (IG1-IG2, IG12-IG13, IG1-IG12). Even for the SESO and

Table 2 **Standard error of a single observation (SESO) and coefficient of variation (CV) concerning absolute and percentage LDF-decrease between the inspiratory gasp tests.**

Inspiratory gasp (number)	SESO		CV	
	LDF decrease		LDF decrease	
	absolute	percentage	absolute	percentage
1 - 2	2 5	12 7	24 3	23 3
12 - 13	3 2	10 6	19 8	18 3
3 - 6	4 1	9 2	40 2	15 7
1 - 12	5 8	10 3	56 0	18 9
9 - 10	4 4	5 6	31 2	9 9

IG-tests 1, 2, 12, 13 *'uncontrolled' IG-tests*
IG-tests 3 and 6 *IG-tests started at end-expiration, inspiration as fast as possible*
IG-tests 9, 10 *IG-tests performed with negative pressure transducer*

CV (between IG3-IG6 and 'uncontrolled' IG-tests) with regard to the percentage LDF-decrease the differences are not significant.

Also the SESO and CV of absolute or percentage LDF-decrease between two IG-tests in which the pressure transducer was used (IG9-10) are not significantly better than between two 'uncontrolled' IG-tests.

Skin temperature (Fig. 2a and 2b)

Despite the fact that the experiments were performed in a climate room, skin temperature remained unstable during the whole experiment. Fig. 2a shows the course of the skin temperature, as the mean (±SD) of all subjects. Fig. 2b shows the skin temperature at the start of the experiment and at the end of the experiment of all 19 subjects. An increase of 0.9 (±0.5) °C was seen during the time of testing

Discussion

Skin blood flow can be measured using laser Doppler fluxmetry (LDF) [25,26]. This non-invasive optical technique uses the Doppler shift of laser light back-scattered by moving red blood cells to estimate the velocity and concentrations of these cells in the tissue volume measured [27]. The laser Doppler signal is related to the relative blood flow in the vascular bed under study. LDF measures mainly AVA shunt flow, when the probe is attached to a region richly supplied with AVA [26,28].

The IG-test can be used to increase sympathetic activity [29,30], resulting in vasoconstriction of the AVA and a decrease in skin blood flow [19,31,32]. This skin vasomotor reflex response can easily be measured using LDF [19]. In diabetic patients the decrease in LDF during the IG-test is diminished compared to normal individuals [21].

Sympathetic nerve fibers are the efferent pathway of this reflex [21], but the afferent pathway is not exactly known. Several decades ago Bolton [31] and Gilliatt [33] examined this reflex using volume plethysmography to measure volume diminution of a digit following a deep breath. They showed that the reflex is independent of the circulatory blood supply [31] and is not initiated by stimulation of a baroreceptor, like the carotid sinus [33]. It is neither dependent on the gaseous composition nor on the temperature of the inspired air [31,33]. They also noticed the relevance of velocity and volume of inspiration on the response. Furthermore Gilliatt showed that the threshold for the reflex response is influenced by the position of the chest and lungs at baseline: an initially expanded position of the chest enhanced the response [33]. Therefore most likely pulmonary mechanoreceptors, i.e. stretch receptors located in or adjacent to the bronchi and bronchioles, are involved [7].

Oberle et al. demonstrated that thermoregulatory mechanisms exert powerful modulatory effects on different cutaneous vasomotor reflexes [22]. To various types of sympathetic stimuli, including the IG-test, warm subjects (skin temperature $> 32°C$) generally responded with vasoconstriction while cold subjects (skin temperature $< 28°C$) reacted with vasodilatation [22].

In accordance with the observations of Oberle et al. [22] in this study in three subjects

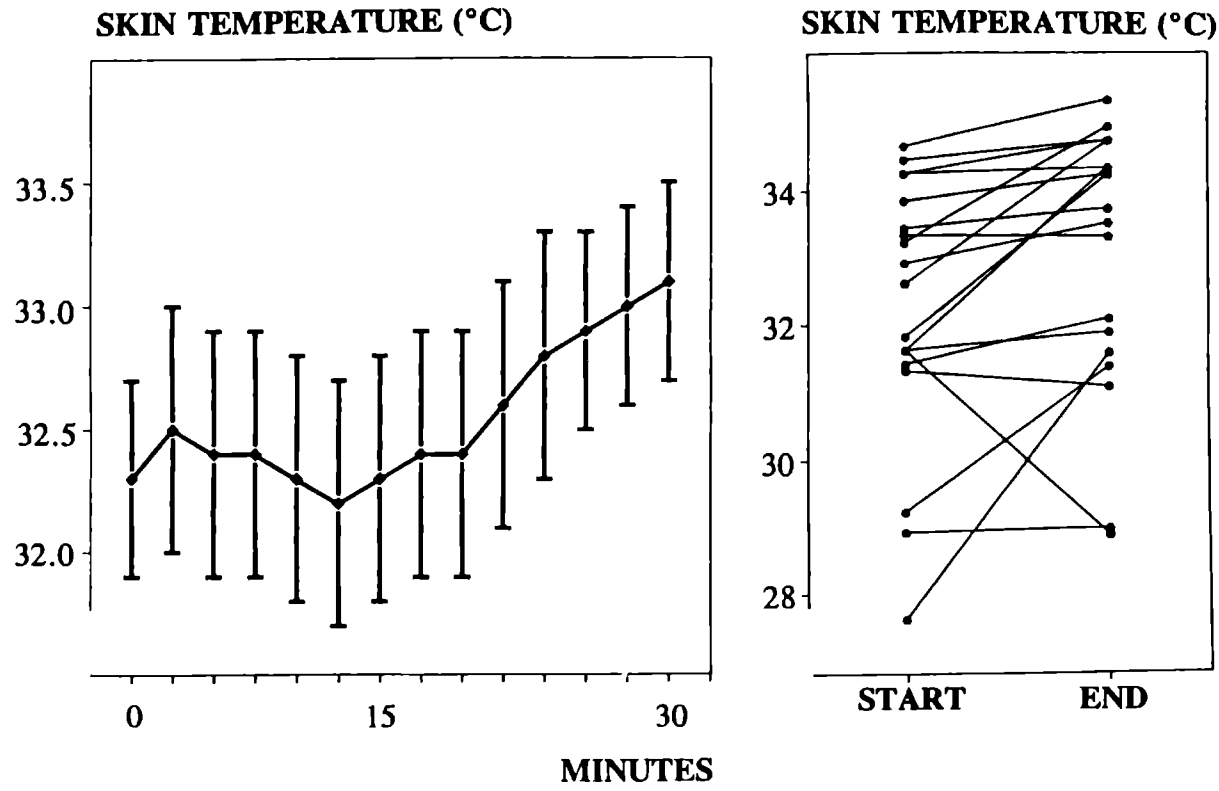

Fig. 2. a) Course of skin temperature (in °C) during the experiment. Mean skin temperature with standard deviation of all subjects plotted.

b) Skin temperature (in °C) of all 19 subjects at the start and at the end of the experiment. The connecting lines show, in most subjects, the increase of skin temperature during the experiment.

with a mean local skin temperature below 28°C either vasodilatation or no vasoconstriction in response to the IG-test occurred. So the exclusion criterium of low skin temperature in these types of studies seems justified.

This study shows that the way in which the IG-test is performed influences the LDF-response. The LDF-decrease is more pronounced when starting the IG-test at end-inspiration and when performing with highest velocity of inspiration. Continuously sucking negative mouth pressure even results in larger LDF-decrease in comparison with taking one deep breath and holding it for ten seconds. However, neither starting the IG-test at end-expiration and performing it with highest velocity of inspiration nor sucking negative mouth pressure improves the reproducibility.

Inspiring a larger volume when starting the IG-test at end-expiration in comparison with starting at end-inspiration and during the inspiration seems obvious. It is striking that starting the IG-test at end-inspiration results in the largest LDF-decrease. This is in accordance with the observation of Gilliatt et al [33].

The observation that higher velocity of inspiration results in a larger LDF-decrease despite smaller inspired volume compared to inspiring with lower velocity, points to a less important effect of the inspired volume on the reflex as opposed to the velocity of inspiration. Moreover, involvement of stretch receptors in the lungs is again suggested with this finding. Taking a breath as fast as possible results in earlier appearance of the lowest value of LDF. This is also in accordance with earlier observations [31,33] of later occurrence of diminution of finger volume when inspiring at a slower rate.

Sucking negative mouth pressure, which correlates well with intrathoracic pressure [24], results in a larger absolute LDF-decrease in comparison with just taking a deep breath and holding it. Application of negative pressure to the upper airways and lungs triggers both in animals and men strong discharges in the sympathetic efferent nerves followed by reflex increase in systemic blood pressure and vasoconstriction [34,35]. Once more this indicates involvement of stretch receptors in the lungs, because these will be stimulated for a longer period if sucking is continued. However performing the IG-test this way is quite exhausting: two subjects had serious and 6 others minor problems with keeping the initially reached negative mouth pressure.

In previous experiments by our group a coefficient of variation (CV) of 39-58% is reported when the test is performed in duplicate on the same occasion [17,19]. Subsequently a heated LDF-probe was used to standardize baseline flux, because skin vasomotor reflexes depend on baseline blood flow Unfortunately this procedure did not improve short-term reproducibility [17]

In this study the coefficient of variation (concerning absolute LDF-decrease) between two 'uncontrolled' IG-tests performed in succession is about 24%, which is smaller than reported in previous studies [17,19]. This is most probably due to the exclusion of subjects with a mean skin temperature below 28°C, as they may not vasoconstrict or even vasodilate in reaction to the IG-test.

Despite the stated influence of the moment in the respiratory cycle at which the IG-test is started and the velocity of inspiration on the LDF-response, there is no improvement of the reproducibility when taking these factors into account This is concluded from comparison of the SESO and CV between the IG3 and IG6, both starting end expiratory and performed with highest velocity of inspiration, with the 'uncontrolled' IG-tests. Even for the SESO and CV of the percentage LDF-decreases the difference is not significant. Unfortunately it is not clear whether this is due to the time passing by between the IG3 and IG6 Sucking negative mouth pressure improves reproducibility, but even this is not significant The CV concerning the percentage LDF-decrease in general is lower than that of the absolute LDF-decrease, evidently because percentage LDF-decreases are corrected for differences in baseline LDF. The CV is negatively influenced by the course of time This is probably (partly) due to the rise in skin temperature during the experiment.

In conclusion, exclusion of subjects with a low skin temperature improves reproducibility of the IG-test. If the aim is to get the largest LDF-decrease during the IG-test, in order e.g. to be able to study the integrity of the peripheral sympathetic system and to discover sympathetic autonomic neuropathy early, the IG-test is best performed with the highest velocity of inspiration starting at end-inspiration or, even better, by sucking continuously negative mouth pressure. The last way of performing the IG-test however is quite exhausting and especially keeping the initially reached negative pressure is difficult, even for young individuals

Reproducibility however did not improve the IG-test by standardization with the above mentioned procedures. Moreover performing the IG-test with the use of a spirometer or negative pressure transducer makes this test more complicated for screening purpose. It seems valid to perform the IG-test in a 'uncontrolled' way, e.g. taking a deep breath and holding it for ten seconds.

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Chapter 6

A clinical comparison of two laser Doppler instruments; Fiber-optic probe versus integrated probe.

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Abstract

Two instruments for laser Doppler fluxmetry were compared; a diode laser (Diodopp) with a He-Ne gas laser (Periflux Pf1d). Spatial variability during baseline registration and temporal variability during 3 standardized provocation tests (suprasystolic occlusion, tilting and inspiratory gasp) were evaluated in 20 healthy volunteers.

The coefficient of variation (=CV) of the registrations on four adjacent places did not show any significant difference between both instruments. The biological zero obtained during 5 min vascular occlusion was always zero with the Diodopp and 4 (3 - 5) (median, [minimum - maximum]) Perfusion Units with the Periflux. The hyperaemic response measured by both instruments was less pronounced during the Diodopp registration (percentage LDF increase 173% (15 - 350) vs 354% (86 - 1100), $p < 0.001$). Although the CV of the LDF parameters obtained by the Diodopp during the standardized provocation tests was almost always lower than with the Periflux, only occasionally the differences reached statistical significance.

We concluded that both laser Doppler instruments are of equal value. The reproducibility of the Diodopp is slightly better, but the hyperaemic response after occlusion is recorded in a different way by both instruments.

Introduction

The laser Doppler technique is a well established non-invasive method to monitor skin blood flow, which has been used in a variety of clinical studies [6,7,10]. However there are some limitations of the technique and pitfalls about the use of laser Doppler fluxmetry (=LDF) that should be taken into consideration. Especially the great temporal and spatial variation in human skin blood flow creates difficulties in microcirculatory blood flow measurement [8].

In this study a diode laser (Diodopp, Applied laser technology, Maarheeze, The Netherlands) was compared with the most widely used He-Ne laser (Periflux PF1d, Perimed, Linköping, Sweden).

Table 1. **Technical data of the laser Doppler instruments used in this study.**

	Diodopp	Periflux Pf1
Laser	diode	He-Ne gas
Maximum output power	5 mW	2 mW
Light output power	2 mW	< 2 mW
Wavelength laser light	780 nm	632.8 nm
Distance of photo detectors	5 mm	2 mm
Cable	flexible	optical fibers
Frequency band	30Hz - 30kHz	20 Hz - 4 kHz or 20 Hz - 12 kHz
Time constant	0.1 ; 0.5 ; 3.0 s	0.2 ; 1.5 ; 3.0 s
Gain	x 0.1; x 0.3; x 1; x 3; x 10.	x 1; x 3; x 10; x 30; x 100

The Diodopp instrument has the laser source and the detectors integrated in the probe and therefore requires no artifact sensitive optical fibers that are easy to damage [4]. Differences in geometry of the detectors, wavelength of the laser light (Table 1) and a higher frequency of the low-pass filter, may result in less spatial and temporal variability of the Diodopp. Therefore both instruments were compared respectively during 3 standardized provocation tests and during measurements on adjacent skin areas.

Methods

Twenty healthy volunteers (14 men and 6 women; mean age 25.5 $\pm$ 2.3 (SD) years) were evaluated during two test sessions. All the subjects gave informed consent to the protocol, that was approved by the local ethical committee.

The tests were performed in a quiet climate room (mean ambient temperature 24.1 $\pm$ 0.4 ^{0}C (SD) and relative humidity of 54 $\pm$ 5%), after 20 min acclimatization in a comfortable supine position. They refrained from smoking for 24 h, from caffeine or alcohol containing beverages for 12 h and from meals for 2 h preceding the tests.

The Periflux LDF was measured in perfusion units (=PU), before the tests this instrument was calibrated using the Periflux motility standard PF100. The Diodopp LDF was measured in relative perfusion units (=RPU), the calibration was performed by the manufacturer using a rotating disk. Therefore and because of probably differences in measured tissue volume under study, the absolute LDF values obtained by both instruments were not compared. After electrical zero calibration the probes were attached to the lateral side of the foot, on a spot where no blood vessels were visible, by means of double sided adhesive tape. The Periflux was adjusted to an upper frequency limit of 12 Khz. The gain and output circuit time constant of both instruments were respectively 1x and 3 sec.

First session

The provocation tests were performed in triplicate, two times in succession on the same day and a third time on a separate occasion. After at least 10 min baseline registration

an ankle cuff was inflated to a suprasystolic pressure to arrest the circulation for 5 min, followed by a sudden deflation. During this postocclusive hyperaemia (PRH) test the following parameters were calculated; mean baseline LDF of 2 min before occlusion, mean LDF during the last minute of occlusion (biological zero [1]), first and maximum LDF peak after occlusion [5], time to the LDF peaks, absolute and percentage LDF increases and the angle in degrees of the tangent of the LDF increase after occlusion. When the LDF returned to a stable baseline, approximately 10 min after deflation of the ankle cuff, a tilt test was performed. The subjects were tilted head-up with an automated tilt table to an angle of 45^0 from horizontal, during 5 min. The table was supplied with a foot support on which the Periflux and Diodopp cable were fixed. Mean baseline LDF during 2 min before tilting, mean LDF during the last minute of the 45^0 position; absolute and percentage LDF decrease were calculated.

The tilt table was then turned back to the horizontal position and after 5 min an inspiratory gasp test was performed. The subjects were asked to take a deep breath as quickly as possible and hold it for 10 s. The test parameters obtained were: mean baseline LDF during 1 min, the lowest value during the gasp and the absolute and percentage LDF decrease. Thereafter the tests were repeated in reversed order. On a separate occasion, 8 (7-15) days later all 3 tests were repeated in the same order. All LDF parameters were corrected for the biological zero, obtained during the PRH test.

In a subgroup of 8 subjects a fourth PRH test was performed with the probes attached to the fingers. A blood pressure cuff was applied around the wrist and inflated up to 300 mmHg during 5 min. In 4 subjects the Diodopp probe was fixed to the volar side of the third fingertip and the Periflux probe to the fourth fingertip and in 4 other persons in reversed order.

Second session

In the same 20 healthy subjects basal LDF measurements on adjacent regions of the lateral foot were performed. The Diodopp and Periflux probe were closely sticked together using a cardboard holder, with four marks. The skin was marked in geometry of the holder marks. After 2 min registration, the cardboard holder was turned 90^0

using the holder and skin marks for location. In this way the probes of the two LDF instruments measured the flux on nearly the same four places. The mean LDF during 2 min and the number of fluctuations of the mean LDF value (=fluxmotion) were calculated for each location.

Statistical analysis

As the data were not normally distributed, results are expressed as median with minimum and maximum values, unless stated otherwise. The coefficient of variation (CV) of the standardized tests was calculated by the formula:

$$\text{CV-S} = \sum \frac{\text{SD (1a and 1b)}}{\text{mean (1a and 1b)}} \times 100\%;$$

$$\text{CV-L} = \sum \frac{\text{SD (1a and 2)}}{\text{mean (1a and 2)}} \times 100\%;$$

in which CV-S means short-term reproducibility, CV-L long-term reproducibility. The symbols 1a and 1b refer to the results of the tests performed on the same day and the symbol 2 refers to the results from the separate occasion. Medians were calculated from these series of CV's, because of the skewness in distribution. In a few subjects the absolute LDF decrease during the tilt test and inspiratory gasp test was zero. In these cases a CV can not be calculated by this formula, because the denominator is zero. The number of subjects used to calculate the median CV of the tests, is noted between brackets in the tables. The CV of the registrations on 4 adjacent skin areas (CV-4) was calculated as follows;

$$\text{CV-4} = \sum \frac{\text{SD (I,II,III and IV)}}{\text{mean (I,II,III and IV)}} \times 100\%$$

The four different places are expressed by I,II,III and IV.
The Wilcoxon signed rank test was used to calculate differences in CV and percentage LDF increase. A p-value < 0.05, two-sided was regarded as statistically significant. NS means not significantly different.

Results

First session

Although the CV of the LDF parameters of the Diodopp during the 3 provocation tests were almost always lower than those obtained with the Periflux, only occasionally the difference reached statistical significance. During the PRH test the short-term reproducibility of the maximum LDF peak and time to the first LDF peak and the long-term reproducibility of the maximum LDF peak differ significantly. The long-term reproducibility of the absolute LDF decrease during tilting and the percentage LDF decrease during inspiratory gasp were significantly better for the Diodopp (Table 2 and 3).
During circulatory arrest the Diodopp always recorded stable zero values, resulting in a nominator in the CV formulas of zero for which reason a CV-S or CV-L can not be calculated. The biological zero of the Periflux was 4 (3 - 5) PU. The angles of the tangent of the LDF response after occlusion were the same for both instruments, but the percentage LDF increase was significantly lower for the Diodopp (Diodopp: 173% (15 - 350) vs Periflux: 354% (86 - 1100), $p < 0.001$). No differences were noted in the percentage LDF decrease during tilting (Diodopp: 43% (0 - 100) vs Periflux: 34% (0 - 150)) and inspiratory gasp (Diodopp 61% (29 - 88) vs Periflux: 50% (0 - 100)).
The LDF results during the PRH test of the fingers were in agreement with the results

obtained from the foot. Also at this location the Diodopp recorded a lower percentage

Table 2. **Short-term (S) and long-term (L) reproducibility of the laser Doppler Flux (LDF) parameters obtained by the two laser Doppler instruments, during a Postocclusive Reactive Hyperaemia (PRH) test.** Reproducibilities are expressed as coefficient of variation (CV).

	CV-S (%)		CV-L (%)	
PRH test	**Diodopp**	**Periflux**	**Diodopp**	**Periflux**
baseline LDF	20.2	20.2	16.7	18.0
biological zero	---#	0	---#	0
first peak LDF	9.5	11.9	14.6	17.7
maximum peak LDF	6.3	11.8	8.1	13.7
time to first peak	28.9	17.4	26.5	18.7
time to maximum peak	16.6	25.4	18.0	21.6
angle of tangent	4.5	2.7	4.6	5.7
LDF increase	8.9	15.6	14.9	14.1
percentage LDF increase	32.2	21.3	18.1	21.5

*# Because the biological zero of the Diodopp was always zero, a CV can not be calculated. Diodopp versus Periflux; * $p < 0.05$, ** $p < 0.01$.*

LDF increase after occlusion (Diodopp: 27% (0 - 146) vs Periflux: 69% (33 - 529), $p < 0.01$). During 60 tilt-tests only 7 artifacts were recorded by the Periflux and none with the Diodopp.

Second session

The CV of the baseline LDF was 14.7% for the Diodopp and 15.8% for the Periflux (NS). The Diodopp recordings showed more fluctuations (12.6 vs 6.8, $p < 0.001$).

Table 3 **Reproducibility of the LDF parameters recorded by the two laser Doppler instruments during tilt-tests and inspiratory gasp tests.** Between brackets the number of subjects in which a coefficient of variation (CV) could be calculated

	CV-S (%)		CV-L (%)		
Tilt-test	**Diodopp**	**Periflux**	**Diodopp**		**Periflux**
baseline LDF	5 6 (n=20)	9 4 (n=20)	17 1 (n=20)		21 9 (n=20)
LDF decrease	28 3 (n=15)	22 3 (n=15)	23 6 (n=19)	*	47 1 (n=19)
percentage LDF decrease	24 4 (n=15)	15 7 (n=15)	30 0 (n=19)		35 2 (n=19)
Inspiratory gasp test					
baseline LDF	7 4 (n=20)	12 3 (n=20)	19 3 (n=20)		33 2 (n=20)
LDF decrease	0 (n=19)	0 (n=19)	28 3 (n=20)		44 4 (n=20)
percentage LDF decrease	10 9 (n=19)	28 3 (n=19)	12 6 (n=20)	**	24 5 (n=20)

*Diodopp versus Periflux, * $p < 0.05$, ** $p < 0.01$*

Discussion

In this study the temporal reproducibility during standardized skin microcirculation tests is slightly better with the Diodopp than with the Periflux Pf1 No major differences were noticed in spatial variability between both instruments During circulatory arrest the biological zero [1] of the Diodopp was always zero There was no difference in percentage LDF decrease during tilting and inspiratory gasp, but after circulatory arrest the

hyperaemic response was significantly attenuated when registered with the Diodopp, both on toes and on fingers.

The small differences in temporal reproducibility and the decreased hyperaemic response by the Diodopp is possibly the result of a higher skin temperature underneath the probe. By using a heated LDF probe the reproducibility of skin vascular provocation tests improves [9]. The reactive hyperaemic response is dependent on skin temperature [3]. In the laser diode of the Diodopp it is necessary to stabilize the probe temperature. This is achieved by mounting the laser on a Peltier element. Without this precaution laser diodes are useless for laser Doppler purposes, because wavelength of the monomode laser is strongly temperature dependent [2]. In case of a high diode temperature the Peltier element cools the laser by giving off heat, which probably results in a higher local skin temperature underneath the probe. To assess the relative heating capacity of the probe we measured, the temperature underneath the Diodopp probe in six persons (data not shown). In 10 min it increased approximately 4^0C. This heat effect was unexpected and probably the result of a combination of an aluminium probe and the cardboard probe-holder, which covered up the skin over a relative large area.

During vascular occlusion the Diodopp always registered zero. Caspary et al reported that the Periflux Pf1d has the highest biological zero value, compared to the other instruments of Perimed (Pf1 and Pf3) [1]. The observed difference in biological zero in this study is most likely the result of a higher electronic off-set point of the Diodopp.

The Diodopp has no probe with artifact sensitive optical fibers. This advantage could be of value in tests were positioning of the region of interest is changed as for example the tilt-test. However if the fiber-probe cable is carefully fixed on the foot support of the automated tilt-table, movements of the cable could be prevented in most cases.

In conclusion the Diodopp and Periflux are instruments of equal value for laser Doppler fluxmetry. The reproducibility of the Diodopp LDF registrations was slightly better, but the hyperaemic response after occlusion was attenuated by this diode laser. Both findings probably result from an increased temperature underneath the Diodopp probe.

Acknowledgements

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Chapter 7

The influence of ulnar nerve blockade on skin microvascular blood flow.

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Abstract

Microvascular research is seriously hampered by the great temporal and spatial variability of the measured skin blood flow and variation in sympathetic vasomotor reflexes within and between persons.

Therefore skin vasomotor reflexes were studied before and after ulnar nerve blockade within the same person, resulting in a temporal complete denervation of the fifth finger and partial denervation of the fourth finger. Skin temperature and laser Doppler flux (LDF) were recorded to measure predominantly arteriovenous shuntflow. Measurements were performed on the palmar tip of the second and fifth finger in 9 healthy volunteers, at baseline, and during a sympathetic reflex test (i.e. inspiratory gasp) and postural response test. Beat-to-beat digital blood pressure was recorded from the third and fourth finger by a Finapres device. Baseline capillary blood cell velocity (CBV) was measured at the nailfold of the second and the fifth finger.

After ulnar blockade baseline skin temperature, LDF and CBV increased significantly, with respectively (mean $\pm$ SE) 3.2 $\pm$ 0.9^{0}C, 20.9 $\pm$ 5.9 relative perfusion units and 0.79 $\pm$ 0.40 mm/sec. The percentage LDF decrease of the fifth finger during inspiratory gasp was 48.2 $\pm$ 5.3% before and 3.1 $\pm$ 0.9% after blockade. The postural response test showed a decrease in LDF of the fifth finger with no significant difference before and after blockade, respectively 12.3 $\pm$ 14.7% and 8.0 $\pm$ 2.7%, while no difference was found in the increase in digital blood pressure in the denervated fourth finger compared to both the same finger before blockade and to the third non-blocked finger.

In conclusion ulnar nerve blockade enables to study sympathetic skin vasomotor reflexes by comparison of a denervated and a non-denervated vascular bed within the same person. After ulnar blockade arteriovenous shunt flow as well as nutritional capillary blood flow increased significantly. Postural vasoconstrictor response is not abolished by ulnar blockade, suggesting that local regulatory mechanisms are more important.

Introduction

The cutaneous microcirculation consists of terminal arteries ($<30\ \mu$ in diameter) and a venous plexus. In between, two types of parallel situated blood vessels are located, i.e. surface capillary loops serving nutritional demands and arteriovenous anastomoses (AVA), essential for body temperature homeostasis [1]. At room temperature skin blood flow by far exceeds nutritional needs; 80 - 90% of total skin blood flow bypasses the nutritional through the AVA [2].

Cutaneous vasculature is predominantly under neural control from a dense sympathetic adrenergic nerve supply, especially in the acral regions. Vasoconstrictor sympathetic skin nerve fibers are the efferent arm of, respectively thermoregulatory reflexes, baroreceptor reflexes, chemoreceptor reflexes and of the reflex response to upright position and exercise [3].

The massive increase in AVA skin blood flow in, for instance, diabetic patients with neuropathy, may compromise the nutritive circulation This hypothesis, known as the capillary steal phenomenon, explains the simultaneous existence of an increased peripheral skin blood flow and trophic skin lesions [4] Yet Flynn et al, found no difference in nutritive blood flow in the toe between patients with and without diabetic neuropathy [5].

A major problem with studies on sympathetic skin vasomotor reflexes is the great temporal and spatial variation of skin blood flow an technical problems of the methods used to study microcirculation Therefore reproducibility of skin vasomotor tests is often troublesome [6-8].

Laser Doppler fluxmetry (LDF) has been widely used in microvascular research [9]. When the LDF probe is attached to an area richly supplied with AVA, considerable evidence suggests that it partially measures AVA flow as well [10,11]. The laser Doppler signal is related to the relative blood flow in the vascular bed of a few square millimetres of exposed skin area Therefore it can not be expressed in millimetres per 100 gram tissue per minute, but only in relative terms [12]. The technique that measures only capillary blood flow in undisturbed vascular beds is television microscopy [13]. Skin temperature, the diameter of the capillaries and limitations of the technique used to

calculate capillary blood cell velocity (CBV) influence the results of capillary microscopy [14].

These, and probably other unknown factors, make it difficult to compare microcirculatory measurements between healthy subjects and patients. Therefore in this study skin microcirculatory reactivity was studied within subjects, before and after proximal ulnar blockade, to compare microvascular changes in a denervated and in a non-denervated vascular bed. This model also enables to elucidate the influence of the nervous system on the flow through the AVA and its consequences for the flow through superficial nutritive capillaries. Furthermore the vasoconstrictor response during postural changes can be studied in order to examine the role of the sympathetic nervous system in skin blood flow regulation.

Methods

Subjects

Eleven healthy volunteers gave written informed consent to the protocol, which was approved by the local ethics committee. None of them smoked or used any medication, except for oral contraceptives (n = 3). There was no history of cardiovascular or pulmonary diseases. All subjects were normotensive, with a supine blood pressure below 140/80 mmHg. Diabetes was ruled out by a HbA1c below 6.4% and a fasting blood glucose below 5.6 mmol/l. To exclude autonomic nervous system dysfunction, five standardized cardiovascular reflex tests were performed with an automated program using a Finapres device [15]. All the test parameters were above the 5th percentile of normal. Subjects were asked to refrain from caffeine- or alcohol-containing beverages for 12 h and from meals 2 h preceding the tests.

Instruments

In this study two Diodopp instruments (Applied Laser Technology, Maarheeze, The Netherlands) were used. This LDF device has the infrared laser source and the detectors integrated into the probe and has been shown to be a suitable instrument for microvascu-

lar research [7]. Both instruments were adjusted to an upper frequency limit of 12 kHz, gain and output circuit time constant were respectively 1x and 0.1 sec. Skin temperature was measured using a Thermocouple (Ellab instruments Copenhagen, Denmark). The digital blood pressure was recorded by two Finapres devices (Finapres model 5; TNO, Amsterdam, the Netherlands). The capillary blood flow in the nailfold of the finger was measured using the technique of television capillary microscopy [14]. The measurement of capillary blood cell velocity (CBV) was performed in individual capillary loops using the video "dual window" technique. This consisted of playing back the video tape through a video densitometer which electronically inserted two video cursors into the video screen viewed on the TV monitor. During the measurements, the densitometer provided analog signal linearly proportional to the light intensity of the area delimited by each video cursor. The video cursors were placed along the length of a capillary, at an upstream position, separated by a known distance. The temporal variations in light intensity measured by the densitometer were then subjected to a real time cross-correlation to determine the transit time of photometric events from one cursor to another. For these calculations a fully computerized system (CapiFlow AB, Kista, Sweden) was used [14].

Study protocol

The tests were performed in a climate room with a constant ambient temperature of 24.2 $\pm$ 0.4 (mean $\pm$ SD) ^{0}C and a relative humidity of 55.0 $\pm$ 0.7%. While the subjects acclimatized in a comfortable supine position for 1 h, the LDF probes were attached to the palmar side of the second and fifth fingertip of the non-dominant hand, by means of double-sided adhesive tape, after electrical zero calibration. The probes of the thermocouple were placed near the LDF probes. The Finapres cuffs were wrapped around the third and fourth finger of the same hand. The hand was fixed to the table alongside the body.

After at least 4 min baseline registration, an inspiratory gasp test was performed. The subjects were asked to take a deep breath as quickly as possible and hold it for 10 s. After at least 2 min of baseline recording a second inspiratory gasp test was performed. After 5 min of baseline registration, the subjects were tilted head-up with an automated

tilt table to an angle of 60^0 from horizontal. After 5 min the table was turned back to the horizontal position and after 2 min of baseline registration all probes were removed. The nailfold capillaries of the second and fifth finger were visualized and video recordings were made during 1 min with a 140 x magnification and during 5 min with a 560 x magnification.

Thereafter, 6 ml of xylocaine 1½ % was injected in the sulcus nervi ulnaris of the elbow. After a few minutes, anaesthesia of the fifth finger and the lateral part of the fourth finger was achieved for several hours. Anaesthesia was tested by determining whether there was absence of sensitivity to a pinprick or light touch. Subsequently the same protocol was repeated.

Before the inspiratory gasp tests and the postural response test skin temperature (in ^{0}C) of both fingers was noted. LDF was measured in relative perfusion units (RPU) [7]. During baseline registration the mean and variability (= maximum - minimum) flux was calculated every minute and averaged for 4 min. The parameters during the inspiratory gasp tests were mean LDF during the last minute of baseline registration, the lowest value during the gasp and the absolute and percentage decrease. During the postural response test the LDF was averaged for each minute of the 5 min baseline registration before the test, for 5 min in the upright position and for 2 min after the test. The percentage postural fall was calculated as mean 5 min baseline LDF before tilting - mean LDF in the 5 min in upright position, divided by the mean 5 min baseline LDF x 100%. Because the Diodopp instrument always records zero during suprasystolic occlusion [7], the LDF signal needs not to be corrected for a biological zero value [16].

The beat to beat digital blood pressure registration with the Finapres devices was recorded by a personal computer and automatically averaged for every 10 s during baseline registration and tilt-test. The blood pressure change was calculated by the mean pressure during 5 min in the supine position minus the mean pressure during the upright position.

The video recordings of the nailfold capillaries in the same regions before and after ulnar blockade were mixed before analysis, to "blind" the investigator. The number of capillaries were counted by using the 140 x magnification and expressed as number per 0.5 mm^2. On the TV screen a rectangle with the size of 1 by 0.5 mm was placed paral-

lel to the nailfold and all the visible capillaries were counted. The CBV was calculated by using the CapiFlow software and the 560 x magnification recordings during 5 min. The filter time constant was 1.0 s and cross correlation limit was above 0.5. The reported CBV is the mean of the 2 - 4 best visible capillaries. The 5 min video recording of these capillaries were used to measure CBV. The measured CBV during 2 min with the lowest (at least less then 15%) artefact percentage was chosen as CBV (artefact excluded). The CBV of each capillary was calculated twice on different occasions and averaged (Coefficient of Variation (CV) in 5 normals was 4.2%). After ulnar blockade the same region of the nailfold was visualized using a striking capillary shape as landmark. In six subjects we succeeded to measure the same capillaries before and after blockade, while in the others measurements were performed in the same region of the nailfold. (CV of CBV measurements of two video recordings of the same capillary in succession in 5 normals was 21.7%).

Statistical analysis

The results are expressed as mean and its standard error (SE), unless stated otherwise. Statistical analysis was performed by Student's t-test for paired samples and Wilcoxon signed rank test when appropriate. A p-value below 0.05, two-sided, was regarded as statistically significant. Correlations were calculated with the Spearman rank correlation coefficient.

Results

In two of the eleven subjects the ulnar blockade was unsuccessful. In one man the second and third finger also showed hyposensitivity and in one woman anaesthesia was not present after two injections of 6 ml xylocaine. The mean age of the 9 (5 ♀) remaining volunteers was 25.4 $\pm$ 3.5 (SD) yrs.

Skin temperature

After blockade of the ulnar nerve the maximum temperature increase of the fifth finger was 3.2 ± 0.9 °C. The temperature of the second finger showed a tendency to decrease (-1.9 ± 1.1 °C). The skin temperature of the blocked finger (35.4 ± 0.2 °C) was significantly higher compared both to the second finger after blockade (29.5 ± 0.8 °C) and to the fifth finger before blockade (31.9 ± 0.9 °C).

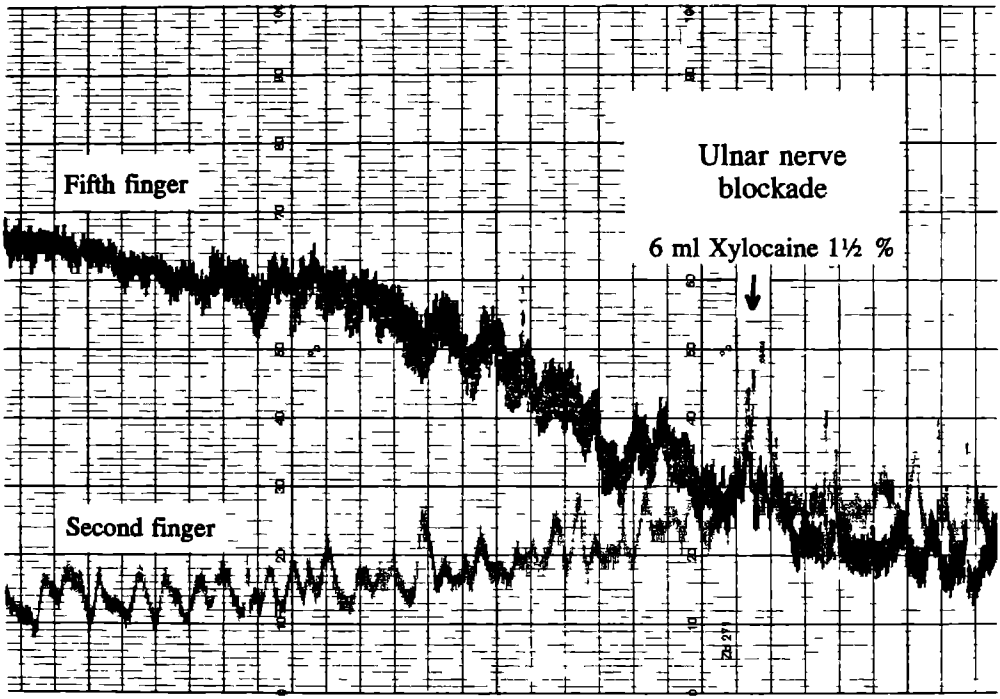


Fig. 1. **LDF registration (from right to left) of the second and fifth finger before and after ulnar nerve blockade.** RPU = relative perfusion Units

Baseline LDF and during inspiratory gasp

Baseline LDF of the fifth finger after blockade was significantly higher and the variation significantly smaller compared to the same finger before blockade and second finger after blockade (Table 1). After blockade the baseline LDF of the fifth finger increased by 20.9 ± 5.9 RPU (Fig. 1).
The variability of the LDF signal decreased by 36.5 ± 3.6 RPU. The baseline LDF of the second finger showed a tendency to drop (-5.6 ± 6.0 PU) after blockade (Table 1).
Before blockade the absolute and percentage LDF decrease during inspiratory gasp was nearly the same in both fingers. After blockade, LDF of the fifth finger decreased minimally during these sympathetic stimulation tests. The LDF decrease of the fifth finger was significantly smaller compared to the decrease before blockade and compared to the second finger after the blockade (Table 1).

Postural vasoconstriction response

Passive tilting results in an increase in mean digital blood pressure of 42.5 ± 2.4 (SD) mmHg. After blockade no difference was found in baseline mean digital blood pressure, nor in blood pressure increase or the area under the curve during tilting between the third and partially denervated fourth finger (Fig. 2).
Before nerve blockade LDF of both fingers decreased in the same way during tilting (Fig 3a); percentage fall in LDF of the second and fifth finger, was respectively 18.6 ± 10.8 and 12.7 ± 13.3 %. After blockade baseline LDF of the fifth finger was higher as mentioned above and tilting still resulted in a significantly ($p < 0.05$) percentage decrease of LDF (Fig 3b). The percentage fall in LDF after blockade of the second finger was 12.3 ± 14.7 % and of the fifth finger 8.0 ± 2.7 %, between the fingers there was no significant difference.

Table 1. **Laser Doppler flux (in RPU) measured from the second and fifth finger, baseline and during inspiratory gasp in duplicate and results of capillaroscopy of the nail-folds, before and after ulnar nerve blockade** (mean ± SE).

	Before blockade		After blockade	
	DIGIT II	DIGIT V	DIGIT II	DIGIT V
LASER DOPPLER FLUX (RPU)				
BASELINE (4 MIN)				
mean	35 4 ± 5 4	46 7 ± 5 1	29 8 ± 4 0	67 7 ± 4 2†*
variability	47 3 ± 24 8	48 3 ± 11 3	43 8 ± 18 3	10 5 ± 6 0‡§
FIRST INSPIRATORY GASP				
absolute decrease	18 4 ± 4 6	23 0 ± 3 4	17 4 ± 4 1	2 0 ± 0 6‡§
percentage decrease in %	50 2 ± 5 1	48 2 ± 5 3	53 9 ± 8 6	3 1 ± 0 9†§
SECOND INSPIRATORY GASP				
absolute decrease	21 3 ± 5 3	22 0 ± 3 5	19 0 ± 3 6	1 4 ± 0 4†§
percentage decrease	47 9 ± 6 6	47 0 ± 4 7	58 2 ± 7 1	2 1 ± 0 6†§
CAPILLAROSCOPY				
NUMBER OF CAPILLARIES (number per 0 5 mm²)	18 9 ± 1 2	16 0 ± 1 0	18 4 ± 0 9	19 0 ± 0 8§
SUBPAPILLARY PLEXUS VISIBLE	1	5	1	8
LENGTH (μm)	122 2 ± 18 5	173 9 ± 27 4	110 5 ± 20 8	173 1 ± 38 9
DIAMETER OF THE CAPILLARIES (μm)				
arteriolar limb	8 8 ± 0 7	9 0 ± 0 6	8 8 ± 0 4	8 4 ± 0 6
venular limb	13 2 ± 1 5	14 4 ± 1 4	11 8 ± 1 0	15 1 ± 1 5
CAPILLARY BLOODCELL VELOCITY (mm/s)	0 26 ± 0 04	0 40 ± 0 07	0 25 ± 0 04	0 99 ± 0 20*‡

‡ *p < 0 05, dig V vs dig II, both after ulnar nerve blockade,* * *p < 0 05, dig V before vs after ulnar nerve blockade,*
† *p < 0 01, dig V vs dig II, both after ulnar nerve blockade,* § *p < 0 01, dig V before vs after ulnar nerve blockade)*

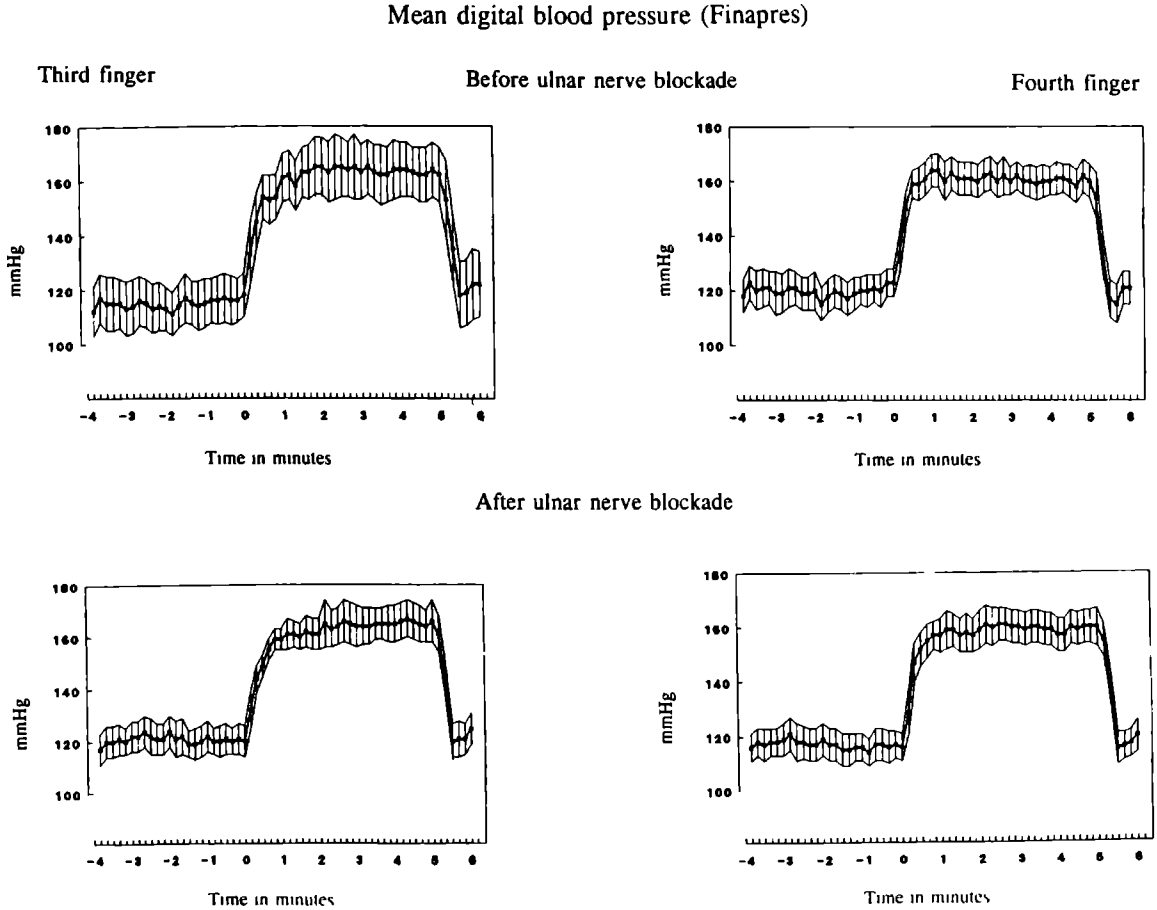
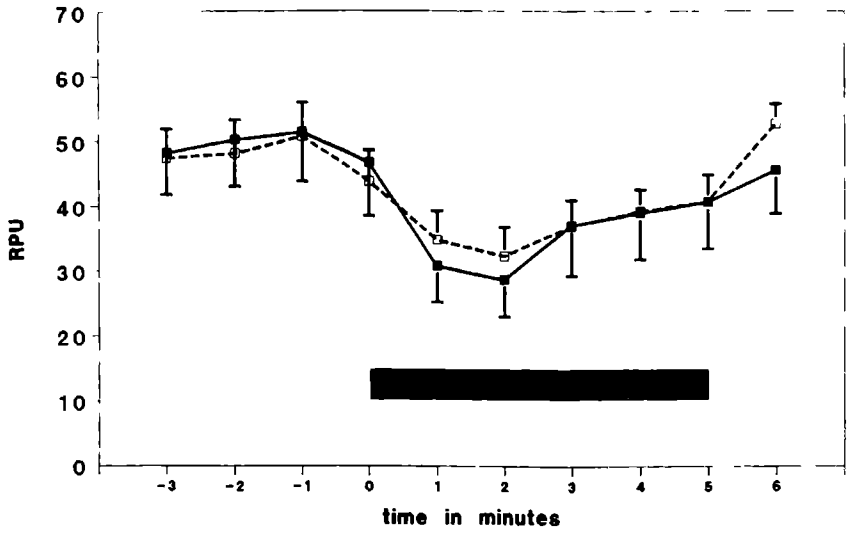


Fig. 2. **Mean arterial pressure (mmHg) measured with a Finapres device from the third and fourth finger during the postural vasoconstriction response test, before and after ulnar blockade** (mean ± SEM).

Before ulnar nerve blockade



After ulnar nerve blockade

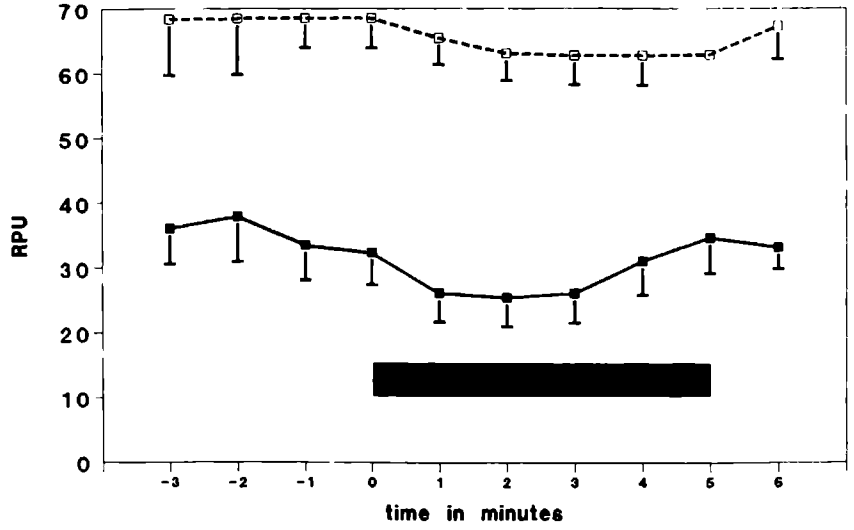


Fig. 3. Laser Doppler flux in RPU (mean ± SE) measured from the second (—■—) and fifth (—□—) finger, before and during 5 min in a 60° from horizontal position of the tilt-table ▬ (postural vasoconstrictor response test), before and after ulnar blockade.

Capillary microscopy

Before blockade less capillaries were seen in the nailfold of the fifth finger compared to the second finger, respectively 16.0 $\pm$ 1.0 and 18.9 $\pm$ 1.2 capillaries/0.5 mm^2 (ns). The capillaries of the fifth finger could be followed over a longer distance, resulting in a difference in measurable length. After ulnar blockade the number of nailfold capillaries in the fifth finger increased significantly, from 16.0 $\pm$ 1.0 to 19.0 $\pm$ 0.8 capillaries/0.5 mm^2 ($p < 0.01$), as did the number of subpapillary plexuses (Table 1). No change was seen in diameter of both limbs or length of the capillaries of the fifth finger, after blockade. However ulnar blockade resulted in a pronounced increase in CBV (0.79 $\pm$ 0.40 mm/sec) of the fifth finger. CBV of the fifth finger after blockade was significantly higher compared to the second finger after blockade ($p < 0.05$) and to the fifth finger before blockade ($p < 0.05$) (Table 1).

Correlations

A significant correlation was found between the increase in baseline skin temperature and baseline LDF ($r = 0.80$, $p < 0.01$) and between the increase in skin temperature and the increase in CBV of the fifth finger ($r = 0.72$, $p < 0.05$), after ulnar blockade.

Discussion

Microcirculatory measurements before and after ulnar nerve blockade enable to establish a pathophysiological model for studying the skin blood flow in a denervated and a non-denervated skin area, within subjects. In this way the role of the sympathetic nervous system and the consequences of sympathetic failure on the regulation of skin blood flow can be studied.

After ulnar blockade skin temperature, LDF, the number of visible nailfold capillaries and CBV of the fifth finger increased. The variability in LDF expressed as range of the LDF signal decreased. No decrease in LDF was noticed during inspiratory gasp, but

LDF still significantly decreased during the postural vasoconstrictor test. Ulnar nerve blockade did not change digital blood pressure response during this test in the partially denervated fourth finger. These results show that the sympathetic nervous system plays a major role in determining baseline skin vascular tone and in skin vascular reactivity towards a deep breath. Its role is minor in skin vascular response towards postural changes.

After blockade of the ulnar nerve both skin temperature and baseline LDF increased significantly and both changes were significantly correlated. A higher skin temperature after ulnar blockade was also found by Lewis and Pickering [17] and an increase in LDF was noticed by Saumet et al after musculocutaneous and median nerve blockade [18]. Release of sympathetic vasoconstrictor tone after nerve blockade results in opening of the AVA and explains the increase of skin temperature and probably the increase in baseline LDF [19,20]. Sympathetic outflow to human skin nerves is increased by an inspiratory gasp [21], resulting in a decrease in skin blood flow [6]. After ulnar blockade LDF no longer decreased during this respiratory manoeuvre.

The LDF method relies both on the penetration and the return of laser light from moving elements within the skin. Only a limited theoretical basis is available for a prediction of the penetration depth of the laser light into the skin. It currently appears that the red and infrared wavelengths, coupled with an appropriate separation of transmitter and receiver provide a larger contribution from deeper dermal structures [22,23], as AVA. By using this LD configuration LDF showed no change when the skin was visibly blanched by topical corticosteroids [11], but when blue laser light was used a detectable reduction in LDF was seen [24]. Synchronous assessment of human skin microcirculation by LDF and capillaroscopy showed discrepancies, which can be interpreted as evidence that LDF records blood flow in vessels in addition to the superficial, nutritional capillaries [10]. For these reasons it is suggested that LDF, used in areas richly supplied with AVA measures partially AVA flow.

After blockade capillary blood flow increased significantly. Both the number of functionting capillaries and CBV increased. The maximum measurable CBV using the CapiFlow software depends on the distance of the two video cursors on the TV-screen (CBV_{max} = cursor distance/0.02). Therefore the length of the capillaries visible on the TV-screen is

important to measure high CBV. Fortunately, the length of the capillaries of the fifth finger were visible over a longer distance then those of the second finger. As shown by others the second finger often has a steep nail wall reducing the visible length of the capillaries under study [25]. Another limitation of the technique is the fact that accurate measurements can easily be performed in capillaries in which plasma gaps are present. In this way bias is introduced in selecting capillaries for measurements of CBV. Capillaries with a high CBV show less or no clear plasma spaces and hence will not be measured accurately. Because of these limitations of the CBV measurements with the CapiFlow system the increase in CBV of the denervated finger is especially underestimated.

The increase in nailfold capillary blood flow after ulnar blockade may be important in understanding the changes in the foot skin microcirculation in diabetic neuropathy. It is hypothesized that the increase in AVA skin blood flow may compromise capillary nutritive blood flow, and be responsible for the healing problems of neuropathic foot ulcers; a hypothesis known as the "capillary steal phenomenon" [9]. The results in the present study are, however, in contradiction with a capillary steal hypothesis.

Flynn et al., using capillary microscopy, found no difference in CBV in the toe nailfold between patients with and without diabetic neuropathy. Because of the increase in erythrocyte column width, an increase in "erythrocyte flux" (calculated from the measured values of CBV and erythrocyte column width, corrected for the duration of stop flow) was found [5]. They concluded therefore that foot skin capillary blood flow is increased in diabetic neuropathy. This increase in diameter of the capillaries is probably the result of long-standing increased capillary blood flow, cq capillary pressure. Recently Sandeman et al., found nail-fold capillary hypertension early in the course of diabetes [26]. Long-standing raised capillary pressure is supposed to induce late structural changes, which ultimately lead to loss of microvascular function and relative under-perfusion, which can result in reduced healing potential of skin following minor injury [27,28]. If the increase in capillary flow due to sympathetic denervation as found in our study, exist in diabetic neuropathic feet, it may in parallel accelerate and compound intrinsic microvascular functional abnormalities.

During postural change no difference in digital blood pressure was found in the partial

denervated fourth finger. The postural vasoconstrictor response of the fifth finger was not abolished by ulnar blockade. This response is probably mediated by myogenic autoregulation at the precapillary level [29,30]. However evidence suggests that vasoconstriction in response to increased hydrostatic pressure is mediated by a local sympathetic axon reflex, which leads to vasoconstriction of the arterioles [31,32]. Local nerve blockade [33], in contrast to blockade at some distance [34] or sympathectomy [35], diminishes the postural vasoconstrictor response. However, the concomitant reduction in flow in both feet when only one foot is lowered below heart level, suggests that a central mechanism is involved too [36]. After lumbar sympathetic blockade, the flow in the horizontal foot remains virtually constant, indicating that the central component is mainly mediated via efferent sympathetic nerves. The present study once more shows that the vasoconstrictor response during tilting is mainly mediated by local neurogenic and/or myogenic mechanisms, partially supplemented by a central component [36].

In conclusion, microcirculatory measurements before and after ulnar nerve blockade enable to study the regulation of the denervated and non-denervated skin microcirculation within persons. This is not only a model to study the consequences of sympathetic failure, as it also creates the opportunity for studying the role of the sympathetic nervous system in the regulation of skin microcirculation in syndromes with sympathetic dysfunction. After blockade the flow through AVA as well as nutritive capillaries increases significantly. In contrast the nervous system plays only a minor role in the vasoconstrictor response to postural changes, because LDF still decreased significantly after ulnar nerve blockade.

Acknowledgements

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Chapter 8

A computerized survey of a diabetic university out-patient clinic, with special emphasis on foot problems.

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Abstract

To monitor the diabetic care process and especially to improve diabetic foot care, data of patients of the diabetic out-patient clinic of the University Hospital Nijmegen were collected.

For this purpose a simple dBase III program was developed and several data, especially concerning diabetic foot complications of 822 diabetic patients were registered.

Of the registered patients 530 were insulin dependent (IDDM) and 292 non-insulin dependent (NIDDM). The mean HbA1c level was 8.7 $\pm$ 1.7%, with no difference between both types of diabetes. Men (379) had a slight, but significant lower HbA1c than women (443), respectively 8.5 $\pm$ 1.5% and 8.8 $\pm$ 1.8% ($p < 0.01$). This difference was most pronounced in the age group under 20 and over 70 years. Hypertension and macrovascular complications were more often present among NIDDM patients. The prevalence of nephropathy was similar in NIDDM and IDDM patients.

Diabetic foot problems were the most common (30.4%) long-term complications of diabetes, especially in NIDDM patients (40,8%). Neurological disorders were more often present (26.9%), than vascular abnormalities (3.8%). In 6.3% foot ulcers existed at the moment of registration or had been present.

With this Dbase III program, data of the out-patient diabetic department of the University Hospital Nijmegen could be registered in a simple and quick way. It raises the opportunity to select patients especially at risk for diabetic foot complications, in an attempt to improve the diabetic care process.

Introduction

When a large numbers of patients with diabetes mellitus are treated, enormous amounts of data can be gathered. To monitor the outcome and process of diabetes care several computer programs have been developed [1,2]. Indeed the World Health Organisation and International Diabetes Federation on Diabetes Care and Research specifically endorse the use of information technology in the promotion and development of optimal health care delivery [3].

With the aid of a personal computer it has become possible to handle data efficiently and use them effectively. In this way the physician can easily gain all sorts of information about his patient population. A regular survey of patients with specific problems can be performed and a yearly evaluation of the diabetic care process is possible. Furthermore, patients can easily be selected for research projects.

Most of the available programs (DiabCare [2,4], Metabase; BAYER, Diabase; NOVO-NORDISK) are very extensive, so it takes much time to repeatedly record all data and enter them into the computer [5]. Not all items of the currently available programs are equally important and when the number of items increases, adherence of the participants often decreases. To overcome these problems we developed a simple computer program in dBase III to register a limited number of clinical data, concerning the type of diabetes, treatment and secondary complications, with special emphasis on persons at risk for foot ulcers.

Registration and input of a record of one patient into the computer takes only several min. With this program patients attending the diabetic out-patient clinic of the University Hospital Nijmegen were registered. The results of the data analysis are reported here.

Methods

All five physicians participating in the diabetic outpatient clinic of the University Hospital Nijmegen, completed a questionnaire (Table 1) of their out-patient population.

Besides name, gender and birth date of the patients, the type of diabetes and year of

onset were registered. Insulin dependent diabetes mellitus (IDDM) was defined as starting insulin therapy within two years after the diagnosis or ketoacidosis in the past. Diabetic patients who did not fulfill these criteria were considered to have non insulin dependent diabetes mellitus (NIDDM). The most recent HbA1c value (reference value 4.8 - 6.4%) registered. Furthermore medication beside hypoglycaemic was noted. Several long-term complications of diabetes mellitus were registered, according to the following criteria.

Diabetic foot complications:

Neurological disorders:

Vibration sense was tested with a tuning fork (128 Hz) at the medial malleolus and dorsal aspect of the big toe. Clearly decreased or absence of vibration sense on at least one location was considered to be abnormal. Tendon reflexes of the knees and ankles were judged to be present or absent. Absence of the reflex on two or more of the 4 locations was defined as abnormal.

Vascular abnormalities:

Peripheral arterial pulsations were recorded by palpation of the dorsal pedal and posterior tibial artery of both feet. Absence of two or more pedal arteries was considered to be abnormal.

Furthermore *foot ulcers* at present or in the past were registered.

Other complications

Hypertension:

Systolic blood pressure above 160 mmHg and/or diastolic above 90 mmHg or use of antihypertensive drugs, at the moment of registration.

Macrovascular complications:

Presence of angina pectoris, myocardial infarction, cardiac failure, intermittent claudication or peripheral vascular surgery and cerebrovascular disease.

Table 1. **Registration form**

Name:	male / female	Registration number	
Birth date	.. / .. / ..		
Date of registration	.. / .. / ..	Dr:	
Diabetes	IDDM		0
	NIDDM		0
Diabetes since			19 ..
HbA1c			... %
Patient uses insulin			0
Other medication	oral hypoglycaemic agents		0
	cardiovascular drugs		0
	sedative medicine		0
	pulmonary medication		0
	anticoagulants		0
Hypertension			0
Macroangiopathy	Ang Pect* / Myocard Inf** / CABG***		0
	Congestive heart failure		0
	Claudication / Vascular surgery		0
	Cerebrovascular disease		0
Retinopathy			0
Nephropathy	Microalbuminuria		0
	Proteinuria		0
	End stage renal disease		0
Neuropathy	Absent vibration sense (toes / ankle)		0
	Absence of reflexes		0
	Absent pedal pulsations		0
	Ulceration (present or in the past)		0
	Amputation		0

** Angina pectoris ** Myocardial infarction*

*** CABG: coronary artery bypass graft.*

Retinopathy:

Presence of retinopathy, documented by the ophthalmologist or treatment with laser-coagulation.

Nephropathy:

An albumin excretion rate (AER) above 20 μg/min, measured on two different occasions, was defined as microalbuminuria. A computer print-out of the laboratory results with all the AER values of the last year, was used to correct or complete the AER results. An AER of more than 200 μg/min or a positive albustix with or without an increased serum creatinine concentration was registered as proteinuria. Patients on dialysis or who underwent a kidney transplantation were registered as having end-stage renal disease.

Statistical analysis

The results are expressed as mean $\pm$ SD, unless stated otherwise. Statistical analyses were performed by Student's t-test for unpaired parametric data, by Wilcoxon rank sum test for non-parametric data and by Chi-square test for binomial data. Results with a two-tailed P value of less than 0.05 were considered to be statistically significant.

Adjusted odds ratios were derived from logistic regression models that included factors as age, duration of diabetes, HbA1c and hypertension.

Statistical evaluation was performed with the statistical computer program package, SAS software (SAS Institute Inc., Cary, USA).

Results

Based on information of the department of medical administration, an estimated 1300 patients who visited the out-patient clinic of the department of internal medicine of the University Hospital Nijmegen, are registered as having diabetes. Approximately 900 patients are regular visitors of the diabetic out-patient clinic. After 11 months 822

patients were registered, with the computer program.

Of these 822 patients, 443 (53.9%) were women and 379 (46.1%) men; 530 (64.5%) had IDDM and 292 (35.5%) NIDDM. The duration of diabetes was longer for IDDM patients than for NIDDM patients (median 18.1 yrs versus 11.9 yrs, $p < 0.001$). NIDDM patients were older (58.3 ± 14.3 yrs versus 38.2 ± 13.8 yrs, $p < 0.001$).

The therapeutic modalities of the patients are shown in table 2. Mean HbA1c of all the recorded patients was 8.7 ± 1.7%. There was no difference between IDDM and NIDDM patients. NIDDM patients on a diet alone had the lowest HbA1c level (7.7 ± 1.7%), while those on both insulin and oral hypoglycaemic medication the highest (9.2 ± 2.0%).

Table 2. **Treatment modalities and corresponding HbA1c values of the registered diabetic patients.**

	Number (%)	HbA1c (%)
Total number of patients	822	8.7 ± 1.7
IDDM	530 (64.5%)	8.7 ± 1.7
NIDDM	292 (35.5%)	8.7 ± 1.7
1. diet only	25 (8.6%)	7.7 ± 1.7
2. oral hypoglycaemic agents	99 (33.9%)	8.4 ± 1.5
3. insulin	151 (51.7%)	9.0 ± 1.7
2 and 3	17 (5.8%)	9.2 ± 2.0

Metabolic control in men was slightly, but significantly better than in women, respectively 8.5 ± 1.5% and 8.8 ± 1.8%, ($p < 0.01$). This was the case for all age categories, except the category 31 - 40 yrs and most pronounced in the age categories under 20 and over 71 year of age (Fig. 1).

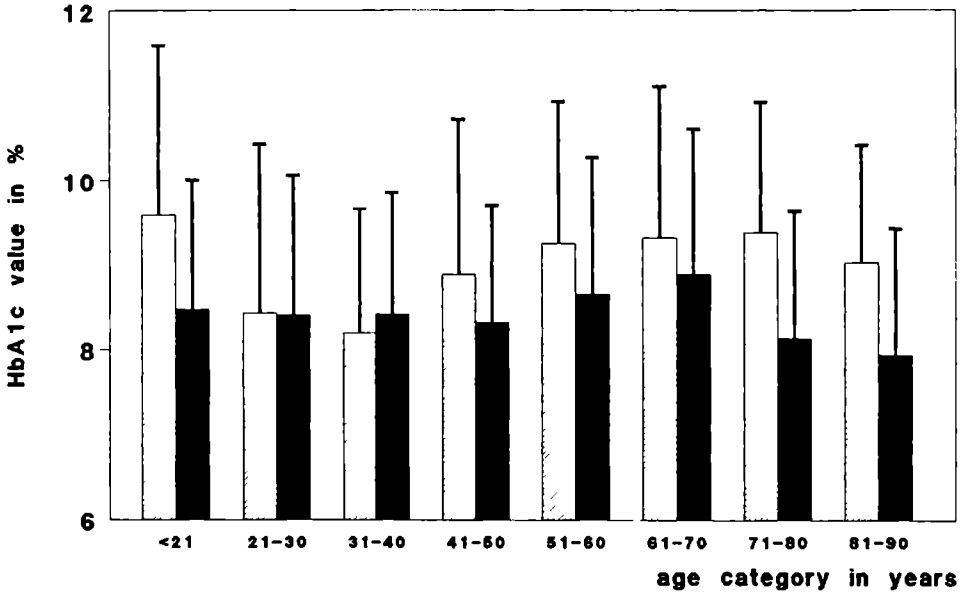

Fig. 1. **HbA1c value per 10 years of age of all diabetic patients.** ▨ **women and** ■ **men.** Mean and SD are shown in the figure.

Of NIDDM patients 152 (52.1%) and of the IDDM patients 72 (13.6%; $p < 0.001$) used co-medication, especially cardiovascular drugs (Table 3).

Diabetic foot complications (Fig. 2)

Of all 822 registered patients, 250 (30.4%) had one or more foot complications as defined (absence of vibration sense and/or reflexes and/or pulsations and or presence of an ulcer at present or in the past). The percentage of foot complications was highest in NIDDM; 119 (40.8%) versus 131 (24,7%); $p < 0.0001$ and foot ulcerations occurred more frequently in NIDDM than in IDDM, respectively 27 (9.3%) versus 25 (4.7%); $p < 0.01$).

Table 3. **Number (percentage) of diabetic patients who used drugs other than oral hypoglycaemic agents.**

Drugs	IDDM	NIDDM
Cardiovascular	52 (9.8%)	122 (41.8%)
Respiratory	8 (1.5%)	16 (5.5%)
Sedative	7 (1.3%)	22 (7.5%)
Anticoagulant	11 (2.1%)	36 (12.3%)

Neurological disorders (absence of vibration sense and/or reflexes) were present in 111 (23.4%) IDDM patients and 110 (37.7%) NIDDM patients ($p < 0.0001$). Also *vascular abnormalities* (absence of pulsations and/or claudication or peripheral vascular surgery) were more often present in NIDDM; 19 (9.9%) versus 12 (2.3%)($p < 0.001$). Among patients with a *foot ulcer* 26.9% had *vascular abnormalities*, while in the group of patients without a *foot ulcer* this was the case in only 3.5%, for *neurological disorders* the results were 84.6% and 24.7% respectively.

Logistic regression analysis of factors associated with *neurological disorders* showed significant Odd's ratios for age and HbA1c level, concerning IDDM and NIDDM patients, while duration of diabetes had only an significant Odd's ratio among IDDM patients. Age was a risk factor for *vascular abnormalities* in both types of diabetic patients and the duration of diabetes only in NIDDM patients. In IDDM patients foot ulcers were associated with the presence of hypertension (Odd's ratio 3.29).

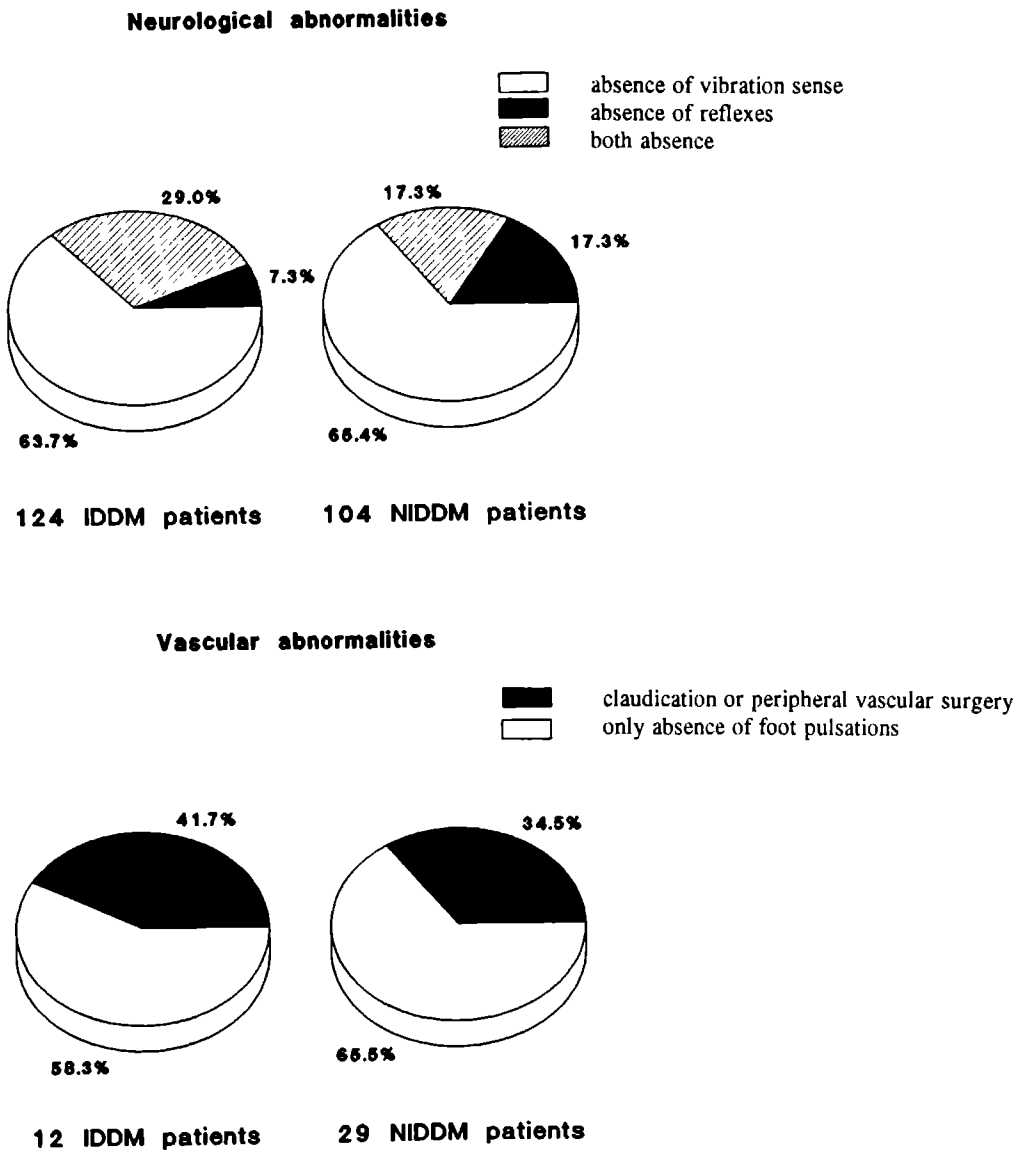

Fig. 2. Neurological disorders (upper part) and vascular abnormalities of 250 diabetic patients with one or more foot complications.

Other complications

Hypertension

According to our definition, 177 (21.5%) patients had hypertension; NIDDM more often than IDDM patients (respectively 100 (34.3%) and 77 (21.5%); $p < 0.001$). Hypertension was associated with age in both IDDM and NIDDM patients, while an association between the existence of hypertension and the duration of diabetes was only established in IDDM (Table 4).

Table 4a. **Significant, adjusted Odd's ratios (95% confidence interval) derived from logistic regression models, concerning IDDM patients.**

	Age	Duration of diabetes	HbA1c (%)	Hypertension
Foot complications				
ulcers	1.46 (1 04 - 2 05)	2.12 (1 40 - 3 22)	1.37 (1 05 - 1 79)	3.29 (1 31 8 22)
neurologic disorders	1.61 (1 32 - 1 97)	2.52 (1 92 - 3 32)	1.26 (1 08 - 1 47)	---
vascular abnormalities	1.95 (1 34 - 2 86)	ns	ns	ns
Macroangiopathy	2.61 (1 88 - 3 61)	ns	ns	ns
Hypertension	1.25 (1 02 - 1 53)	1.52 (1 19 - 1 96)	ns	---
Microalbuminuria	ns	1.47 (1 14 - 1 89)	1.23 (1 04 - 1 47)	ns
Proteinuria	0.61 (0 46 - 0 82)	2.89 (1 07 - 1 15)	1.22 (1 10 - 1 47)	---

ns: not significant

Table 4b. **Significant adjusted Odd's ratios (95% confidence interval) derived from logistic regression models, concerning NIDDM patients.**

	Age	Duration of diabetes	HbA1c (%)	Hypertension
Foot complications				
ulcers	1.58 (1 07 - 2 32)	1.60 (1 01 - 2 53)	ns	ns
neurological disorders	2.09 (1 62 - 2 69)	ns	1.22 (1 06 1 45)	---
vascular abnormalities	1.91 (1 27 - 2 87)	1.94 (1 22 - 3 09)	ns	ns
Macroangiopathy	2.17 (1 63 - 2 89)	ns	ns	ns
Hypertension	1.32 (1 09 - 1 59)	ns	1.16 (1 01 1 35)	---
Microalbuminuria	ns	1.34 (1 04 - 1 71)	ns	ns
Proteinuria	ns	2.07 (1 39 - 3 10)	ns	---

ns = not significant.

Macrovascular complications

NIDDM patients had more often macrovascular complications than IDDM patients (55 (18.8%) versus 23 (4.3%); $p < 0.001$). Age was associated with cardiovascular complications for both types of diabetes (Table 4).

Retinopathy

Because information from the ophthalmologist was often absent, retinopathy was almost certainly underestimated and therefore excluded from further analysis.

Nephropathy

All the registered microalbuminuria results were verified using a laboratory data base. Therefore it was known wether this test was performed in a patient or not. Microalbuminuria was less frequently determined among older NIDDM patients (Table 5).

There was an association between the duration of diabetes and microalbuminuria in both IDDM and NIDDM patients, while the association between HbA1c level and the presence of microalbuminuria only existed in IDDM (Table 4). Because diabetic nephropathy results in hypertension, we did not calculate Odd's ratio's for this parameter.

Table 5. **Number (percentage) of patients with diabetic nephropathy.**

	IDDM		NIDDM	
	Present	**Unknown**	**Present**	**Unknown**
Microalbumuria	48 (9.1%)	27 (5.1%)	22 (7.5%)	43 (14.7%)
Proteinuria	73 (13.8%)	--	32 (11.0%)	--
End-stage renal disease	12 (2.3%)	--	8 (2.8%)	--

Discussion

In this study we show the successful implementation of a simple database in an university diabetic out-patient clinic, which is of course no reflection of the general diabetic population. With the aid of this Dbase III program it was possible to register all desired data efficiently and the results could be analyzed effectively. The data analysis creates an opportunity to evaluate and improve the provided diabetes care, furthermore selection of patients for research projects is facilitated.

The diabetes care delivery is improved in several ways. When completing the questionnaire, vacancies in diabetes care were easily detected and hence corrected by the diabetologist, before the data were put into the computer. In this way specific problems inevitably can get more attention. Patients at risk of foot ulceration can easily be selected for specific foot care education, which is of proven value in preventing ulceration [6]. Furthermore groups of patients who need special care, can be detected. As an example, young female IDDM patients showed in general a poor metabolic control, as did NIDDM patients treated with the combination of insulin and oral hypoglycaemic treatment. The poor metabolic control in the latter was also found by Wolffenbuttel et al, among 124 NIDDM patients [7]. The database is probably more effective when it is regularly updated and repeatedly analyzed, so that improvements in diabetes care and possible changes in appearance of late diabetic complications can be evaluated [8].

As expected, in NIDDM patients macrovascular complications, hypertension and the use of cardiovascular drugs were more frequently present compared to IDDM patients, while the prevalence of nephropathy was the same [9]. In our hospital, patients with end-stage renal disease are primarily controlled by the nephrologist in collaboration with the diabetologist, therefore the number of patients with end-stage renal disease probably is underestimated.

In the population under study approximately 30% were more or less at risk to develop a foot ulcer, because one or several indicators of diabetic foot problems were present. This percentage strongly depends on the number of risk factors used for selection and the description criteria employed. For example foot deformities or trophic skin lesions were not registered.

There are limitations in the way neurological disorders are described. Vibration sense and reflexes deteriorate with age [10], and their absence is therefore not perse a sign of diabetic neuropathy [11]. In accordance with this, logistic regression showed an association of age with neurological disorders for both NIDDM and IDDM patients. The diagnosis of peripheral sensorimotor neuropathy can be made on the basis of symptoms, signs, quantitative sensory test instruments and electrodiagnostic studies. In a previous study we showed the limited value of some sensory function tests, because of high variability and poor reproducibility [12]. Electrodiagnostic studies must be considered too

expensive and time-consuming for a screening purpose. Although different diagnostic criteria were used, the percentage of patients with neuropathy and the predominance of NIDDM patients with this complication, found in the population surveyed, are comparable with the results of a recent multicentre study [13]. Neurological disorders were seen more often than vascular abnormalities, which emphasizes the major role of neuropathy in the aetiology of a diabetic foot [14]. However patients with an ulcer more often had macrovascular abnormalities

In 52 (6.2%) patients a foot ulcer was present in the past or at the moment of registration. This is comparable with other population based studies, reporting percentages of 4 to 12% [15,16]

Surprisingly a positive association between hypertension and a foot ulcer (Odd's ratio 3.29, $p < 0.001$) was found in IDDM patients, but not in NIDDM patients In IDDM patients [17] and in patients with hypertension [18] an increase in nailfold capillary pressure is found, which is not the case in NIDDM patients [19] This increase in capillary pressure may lead to structural changes in the microcirculation, disturbing its function [20]. This could explain the positive association between the presence of hypertension and foot ulcers in IDDM patients.

In summary: with this simple dBase III program we were able to register most of the desired data, especially concerning diabetic foot problems. Furthermore, analysis of these data creates the opportunity to monitor the provided diabetes care, in an attempt to improve it

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Chapter 9

Skin microcirculation of the foot in diabetic neuropathy

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Submitted

Abstract

In the diabetic neuropathic foot, total skin blood flow is increased due to an increased shuntflow. The question is if this increased anastomotic shuntflow leads to either under or overperfused nutritive capillaries.

To solve this question, skin microcirculation tests of the left big toe were performed in 20 healthy controls and in 40 insulin dependent diabetic patients without macroangiopathy, 20 without and 20 with neuropathy. Skin temperature measurements and laser Doppler fluxmetry (LDF) were performed to record mainly shuntflow and capillaroscopy to study nailfold capillary blood flow.

The IDDM patients with neuropathy had a higher baseline skin temperature (mean $\pm$ SEM; 30.0 $\pm$ 0.6 ^{0}C) and LDF (26.2 $\pm$ 2.2 Perfusion Units), compared to patients without neuropathy (27.2 $\pm$ 0.8 ^{0}C, $p < 0.01$; 16.1 $\pm$ 2.0 PU, $p < 0.01$) and healthy controls (27.9 $\pm$ 0.7, $p < 0.05$; 18.6 $\pm$ 2.8 PU, $p < 0.05$). Sympathetic stimulation (inspiratory gasp) resulted in a smaller LDF decrease in the neuropathic patients (31.4 $\pm$ 4.6%) compared to non-neuropathic patients (48.2 $\pm$ 5.1%; $p < 0.05$) and controls (49.0 $\pm$ 3.8%; $p < 0.05$), while no difference between the three groups was seen in the LDF decrease during a postural vasoconstriction test. The number of visible capillaries was the highest in the neuropathic patients (10.2 $\pm$ 0.6 /0.5 mm^2), when compared with non-neuropathic patients (8.7 $\pm$ 1.2/0.5 mm^2, $p < 0.05$) and controls (8.3 $\pm$ 0.3/0.5 mm^2, $p < 0.001$). Capillary bloodcell velocity was significantly higher in the neuropathic patients (0.32 $\pm$ 0.05 mm/s), compared to the non-neuropathic patients (0.23 $\pm$ 0.03 mm/s, $p < 0.05$) and controls, (0.23 $\pm$ 0.02 mm/s, $p < 0.01$).

We conclude that there is an overperfused nutritive capillary circulation in the diabetic neuropathic foot. This is in contradiction with a capillary steal-phenomenon and favours the hyperdynamic hypothesis to explain the decreased healing potential in diabetic neuropathic foot ulceration.

Introduction

One of the most serious long-term sequelae of diabetes mellitus is the diabetic foot complicated by trophic skin lesions [1]. These may develop in spite of an increased total skin blood flow [2-4]. This increase in flow is supposed to be related to peripheral sympathetic denervation, resulting in an increased flow through the arteriovenous anastomoses (AVA) [5,6]. These AVA are essential for body temperature homoeostasis. Exposure to cold results in selective vasoconstriction [7]. At room temperature 80-90% of total skin blood flow passes through the AVA and so bypasses the more superficial localized nutritional capillaries [7]. The massive increase in AVA skin blood flow in patients with peripheral autonomic neuropathy due to diabetes, may compromise the nutritive circulation, a hypothesis known as the capillary steal phenomenon [8]. Using an opentipped platinum electrode in the skin of the lower extremities of normal subjects, it was found that preganglionic sympathetic denervation resulted in a decrease in skin oxygen tension. In the presence of an increased total skin blood flow, this suggests a decrease in capillary blood flow after denervation [9]. However in a previous experimental study in healthy volunteers, using capillary microscopy, we have found an increase in capillary bloodcell velocity (CBV) of the fifth finger after temporarily ulnar nerve blockade [10]. But in the one and only clinical study by Flynn et al, no change in the CBV of the toe nailfolds was found in patients with diabetic neuropathy. There was however an increase in the estimated "capillary volume flow" in these diabetic patients, because of the increase in erythrocyte column width [11]. The results of this clinical study argue against capillary underperfusion distal to high anastomotic shuntflow.

In patients with diabetic neuropathy a red foot skin and venous distension are visible especially in the dependent position [12], as a result of a diminished venoarteriolar reflex [13,14]. It has been postulated that this is caused by sympathetic neuropathy [13]. In contrast we have found no difference in postural vasoconstrictor response of the blocked fifth finger after ulnar nerve blockade [10]. Further evidence suggests that this vasoconstriction is mediated by a local sympathetic axon reflex, only partially supplemented by a central component [11,15,16]. Finally the fall in blood flow may also be explained by myogenic autoregulation at the precapillary level [17].

In the present study we carefully selected diabetic patients with and without neuropathy, but without signs of macroangiopathy. Neuropathy was scored and microcirclation tests of the big toe were performed under standardized conditions, to elucidate the influence of diabetic neuropathy on nutritive capillary skin blood flow and on the postural vasoconstrictor response.

Methods

Subjects

Using a database of 1054 diabetic patients controlled at the outpatient clinic of the University Hospital Nijmegen, 40 patients (age: 20 - 65) with insulin dependent diabetes (10 or more years duration), were selected. All were without clinical signs of macroangiopathy, none had hypertension (blood pressure below 160/90 mmHg), nor used medication except for insulin or oral contraceptives. HbA1c level had to be below 10% (normal < 6.4%).

Of these 40 patients 20 showed absence of vibration sense on two or more of the four standard places (dorsal aspect of the big toes and ankles) tested on both feet and absence of tendon reflexes of both knees and/or both ankles. The other 20 selected patients had no signs of neuropathy. Furthermore 20 healthy, age and sex - matched, volunteers were selected as controls, by a newspaper announcement. None smoked or used more than 2 alcohol consumptions a day. All had a normal ECG, normal renal function (creatinine < 110 μmol/l for men and < 90 μmol/l for women) and their total cholesterol level was below 6.5 mmol/l. To exclude macroangiopathy of the lower legs the ankle/brachial index should be $\geq$ 0.9 and toe systolic blood pressure > 100 mmHg [18]. The subjects were asked to refrain from caffeine- or alcohol containing beverages for 24 h and from meals one hour preceding the tests. None had foot ulcers at the time the tests were performed. All subjects gave informed consent to the protocol, that was approved by the local ethics committee.

Study protocol

All subjects were studied in an environmentally controlled room maintained at a temperature of 24.0 ± 0.4 ^{0}C (mean ± SD) and a relative humidity of 55.0 ± 2.2 %. Five cardiovascular autonomic reflex tests according to Ewing were performed with an automated computer program using a Finapres device [19]. Cardiovascular autonomic neuropathy was considered to be present if 2 or more of the 5 tests parameters were below the 5th percentile of the normal. The five test parameters were: 1) The mean difference between the highest heart rate during inspiration and the lowest during expiration for six consecutive forced breathings: *I-E difference*. 2) The difference between the maximum heart rate after standing up and the control heart rate before: ▲ *HRmax*, and the quotient of maximum heart rate after the manoeuvre and the minimal heart thereafter: *T/B ratio*. 3) Difference between mean diastolic blood pressure 50 to 80 s after standing up and during the supine position: ▲*BPdias*. 4) Highest heart rate divided by the lowest heart rate after the Valsalva manoeuvre: *Valsalva ratio*. 5) Highest increase in average diastolic blood pressure over 5 s during 3 min sustained handgrip: *delta BPdias*) [19].

Thereafter peripheral neuropathy was assessed by a neurological disability score, and by measurements of both current perception threshold in milli-amperes (Neurometer^R, Minimed technologies, Sylmar, CA, USA) and by vibratory perception threshold in microns (Vibrameter IV^R, Somedic, Stockholm, Sweden) [20]. The neurological disability score is based on testing lower leg reflexes and gnostic (tuning fork and fine touche) and vital (pricking pain) sensitivity. Maximal score is 48 points [20]. Current perception threshold was obtained of both big toes, for the three frequencies (5, 250, 2000 Hz). Vibratory perception was tested at the metatarsal phalangeal joint of the big toes and at the lateral ankle of both feet.

Subsequently the subjects were situated in a comfortable supine position. Laser Doppler fluxmetry (LDF) (Periflux Pf1d, Perimed, Linköping, Sweden) was used to measure capillary and arteriovenous blood flow [21]. The Periflux was adjusted to an upper frequency limit of 12 kHz and the output circuit time constant and gain were respectively 0.2 and 3x. LDF was measured in perfusion units (PU) [23]. The LDF probe was attached to the plantar surface of the left big toe by double sided adhesive tape. Skin temperature of the toe was measured using a thermocouple (Ellab instruments, Copenha-

gen, Denmark).

During 10 min both instruments measured baseline LDF and skin temperature. Thereafter the feet were warmed with an electric blanket, if skin temperature was below 28°C, before the microcirculation tests were performed in an attempt to standardize cutaneous thermoregulatory mechanisms which exert powerful modulatory effects on skin vasomotor reflexes [23].

After 2 min registration with a skin temperature above 28°C the first inspiratory gasp test was performed. The subjects were asked to take a deep breath as quick as possible and hold it for 10 s. Outflow to sympathetic skin nerves is increased by an inspiratory gasp [24], resulting in a decrease in skin blood flow [25]. After 2 min baseline registration, a second inspiratory gasp test was performed. The parameters during these tests were mean LDF during the last minute of the preceding baseline registration and the absolute and percentage decrease.

After the inspiratory gasp test, the postural vasoconstrictor response was tested. After 5 min baseline registration, the subjects were tilted head-up with an automated tilt table to an angle of 90° to horizontal. 5 Min later the table was turned back to the horizontal position. During this test LDF was averaged for each minute of respectively the 5 min baseline registration, the 5 min postural challenge and the 2 min thereafter.

Prior to the postocclusive hyperaemia test (PRH-test) baseline LDF was again recorded for 5 min. A toe cuff was inflated to a suprasystolic pressure to arrest the circulation for 5 min, followed by a sudden deflation. During the last minute of circulatory arrest a biological zero was determined and subtracted from all previous LDF measurements [26]. Approximately 20 min after this PRH test, the LDF and temperature probe were removed.

To measure capillary nutritive flow, capillaroscopy of the nailfold of the left big toe was performed, while the subjects were in a sitting position. During 1 min videorecordings were made (140 x magnification) to count the number of capillaries. To measure capillary bloodcell velocity (CBV) in two or three capillaries during 5 min (560 x magnification), a computerized system (CapiFlow AB, Kista, Sweden) was used [27]. A full description of the test procedure has been given previously [10]. The videorecordings were mixed to blind the investigator.

Statistical analysis

The results are expressed as mean and standard error of the mean (SEM), unless stated otherwise. Statistical analysis was performed by Wilcoxon signed rank test and Student's t-test for paired samples when appropriate. A two-sided p-value below 0.05, was regarded as statistically significant.

Results

Subjects

The characteristics of the study population are shown in table 1. The duration of diabetes was significantly longer for the diabetic patients with neuropathy. In this group the supine systolic blood pressure was higher compared to the others, while diastolic blood pressure was only higher compared to the diabetic patients without neuropathy. Baseline heart rate in the neuropathic group was significantly increased compared to the control group, but there was no difference between the patients with and without neuropathy. Microvascular diabetic complications as retinopathy and incipient nephropathy were more often present among patients with neuropathy.

Neuropathy (Table 2)

The diabetic patients with neuropathy had of course the highest neurological disability score. The thresholds of current and vibratory perception were significantly worse in the neuropathic group, while there was no difference between normals and diabetic patients without neuropathy. Cardiovascular autonomic neuropathy tests were more often disturbed in the patients with neuropathy, especially the tests based on of heart rate variability.

Only one of the patients without neuropathy had two abnormal autonomic function test results (heart rate response during forced breathing and valsalva manoeuvre), while 2 or more abnormal test results were found in 19 of the patients of the neuropathic group.

Table 1. **Characteristics of the study population** (mean ± SD).

	CONTROLS		DIABETIC PATIENTS		
			With neuropathy		Without neuropathy
Age (yrs)	46.4 ± 10.6		47.9 ± 9.3		43.3 ± 9.9
Male / female	8 / 12		9 / 11		8 / 12
Duration of DM (yrs)			31.5 ± 11.6	**	17.8 ± 5.7
Total daily insulin dose (IU)			43.0 ± 15.4		54.1 ± 18.4
Supine blood pressure (mmHg)					
Systolic	119.3 ± 13.6	**	141.6 ± 13.9	***	118.2 ± 10.1
Diastolic	71.9 ± 7.2		76.5 ± 6.5		69.8 ± 8.2
Baseline heart rate (beats/min)	64.5 ± 11.4	*	74.8 ± 13.2	*	69.0 ± 12.3
$^{Ankle}/_{Brachial}$ index	1.1 ± 0.1		1.1 ± 0.1		1.2 ± 0.1
Blood pressure left toe (mm Hg)	132.9 ± 22.6		136.4 ± 19.3		127.8 ± 20.0
Retinopathy			13		2
HbA1c (%)	5.2 ± 0.3		8.9 ± 1.0		8.4 ± 1.1
Glucose (mmol/l)	5.2 ± 0.6		10.4 ± 4.6		9.3 ± 5.1
Cholesterol (mmol/l)	5.3 ± 1.0		5.6 ± 0.9		5.3 ± 1.0
Creatinine (μmol/l)	76.6 ± 7.3	**	84.3 ± 9.5	**	75.6 ± 8.7
Microalbuminuria (AER 20-200 μg/min)			7		2

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table 2. **Results (mean ± SEM) of the peripheral nerve function tests and number of abnormal cardiovascular reflex tests.**

	Controls		Diabetic patients		
			With neuropathy		Without neuropathy
Neurological disability score	0.8 ± 0.3		24.6 ± 1.9		1.1 ± 0.3
Current perception threshold (in milli-ampere)					
2000 Hz	372.7 ± 19.5	****	763.0 ± 54.8	***	352.6 ± 15.8
250 Hz	175.3 ± 12.0	***	450.0 ± 62.8	***	179.0 ± 11.9
5 Hz	108.6 ± 10.8	**	317.6 ± 57.9	**	126.8 ± 10.0
Vibratory perception threshold (in microns)					
metatarsal	1.8 ± 0.4	***	48.2 ± 11.2	***	1.4 ± 0.3
malleolar	0.9 ± 0.1	***	21.3 ± 7.0	***	0.7 ± 0.1
Cardiovascular reflex tests					
More than 2 abnormal test results	0		19		1
forced breathing	3		17		5
standing up; *heart rate response*	0		12		0
standing up; *blood pressure response*	0		5		0
Valsalva manoeuvre	1		17		1
sustained handgrip	0		4		0

** $P < 0.01$ *** $P < 0.001$ **** $P < 0.0001$

Baseline skin temperature and LDF (Table 3)

Baseline skin temperature and LDF, before warming up were significantly higher in the group with neuropathy, compared to the healthy volunteers as well as diabetic patients without neuropathy

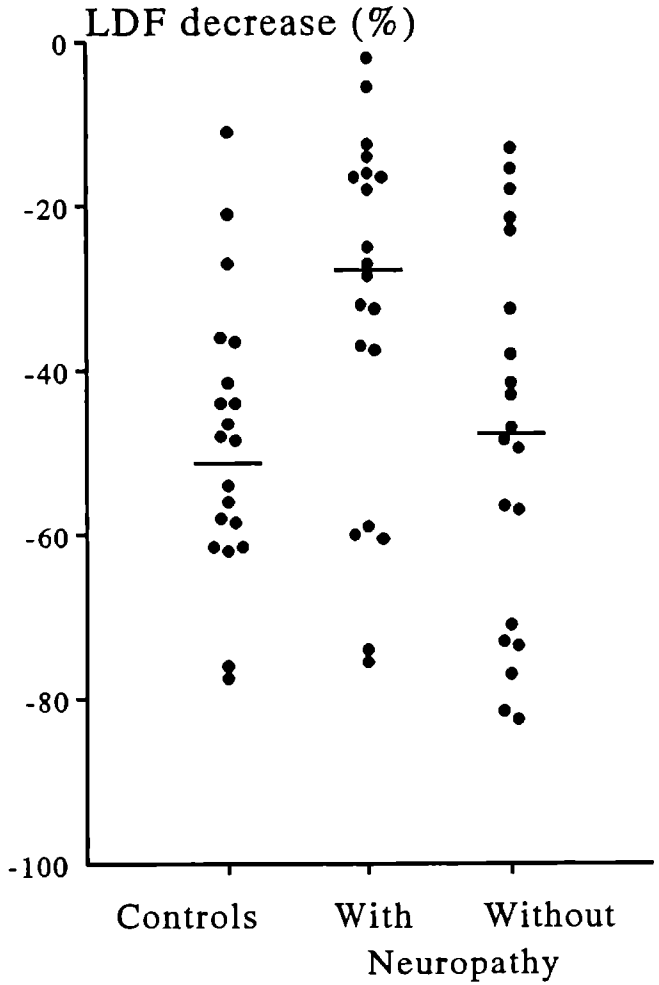

Fig 1 **Mean percentage LDF decrease during two inspiratory gasp tests** (individual test results, median and minimum - maximum)

Inspiratory gasp (Fig 1 and Table 3)

Because baseline skin temperature was below 28°C in 12 normals, 4 neuropathic patients and 11 non-neuropathic patients, the foot skin was warmed up prior to a duplicate inspiratory gasp test. Median (minimum - maximum) percentage LDF decrease was significantly lower in the group of patients with neuropathy 27.8 % (0.0 - 66.5) compared to patients without neuropathy (47.8 % (13.0 - 82.5); $p < 0.05$) and healthy volunteers (51.3 % (11.0 - 77.5); $p < 0.05$). The individual results of the patients with neuropathy showed a somewhat bipartite distribution. Five of the neuropathic patients had a percentage LDF decrease of more than 60%, while in the all others is was less than 40%.

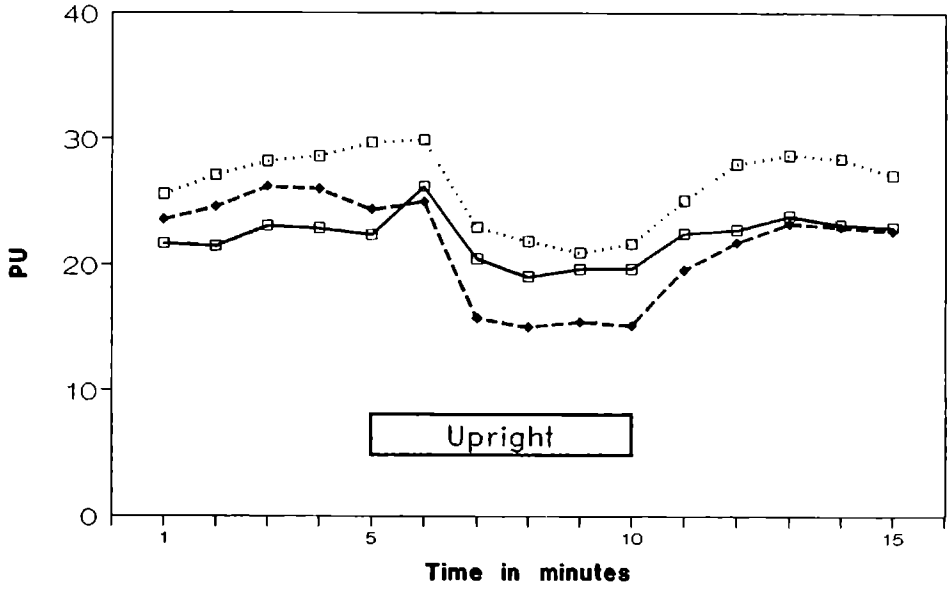

Fig 2. **LDF (mean of each minute registration in PU) during a postural vasoconstriction test in controls (–•–), diabetic patients with neuropathy (··□·) and without neuropathy (—□—).**

Table 3 **Results microcirculation tests** (Mean ± SEM)

	Controls		Diabetic patients		
			With neuropathy		Without neuropathy
Skin temperature (°C)	27 9 ± 0 7	*	30 0 ± 0 6	**	27 2 ± 0 8
Laser Doppler Fluxmetry					
Baseline LDF (PU)	18 6 ± 2 8	*	26 2 ± 2 2	**	16 1 ± 2 0
LDF decrease during inspiratory gasp					
Percentage (%)	49 0 ± 3 8	*	31 4 ± 4 6	*	48 2 ± 5 1
Absolute (PU)	10 1 ± 1 4		7 9 ± 1 2		9 4 ± 1 3
LDF decrease during tilt test					
Percentage (%)	16 0 ± 14 6		17 8 ± 10 4		-0 8 ± 14 2
Absolute (PU)	7 1 ± 2 9		4 4 ± 3 6		1 0 ± 3 2

** P < 0 05 ** P < 0 01*

Table 4. **Results of capillaroscopy of the nailfold of the first left toe.**

	Controls		Diabetic patients		
			With neuropathy		Without neuropathy
Number of visible capillaries (n/0 5 mm^2)	8.3 ± 0.3	***	10.2 ± 0.6	*	8.7 ± 1.2
Length (μm)	137.0 ± 6.9	*	110.6 ± 8.6	*	145.2 ± 10.2
Diameter of the capillaries (μm)					
arterial limb	8.0 ± 0.8		8.1 ± 0.6		7.5 ± 0.5
venular limb	10.5 ± 1.1		9.8 ± 0.8		9.6 ± 0.5
Capillary bloodcell velocity (mm/s)	0.23 ± 0.02	**	0.32 ± 0.05	*	0.23 ± 0.03

*$P < 0.05$ **$P < 0.01$ ***$P < 0.001$

Postural vasoconstrictor response test (Fig. 2 and Table 3)

No difference was seen in LDF response during postural changing between the groups. Percentage LDF decrease in the diabetics with and without neuropathy and controls were respectively (median (minimum - maximum)) 41.1% (-166.7 - 80.0), 28.0% (-117.5 - 77.5) and 14.7% (-138.5 - 73.7).

Capillary microscopy (Table 4)

The number of visible nailfold capillaries and CBV were significantly higher in the diabetic neuropathy group. The capillaries of the neuropathic patients were visible over a smaller distance than those of the other groups. No differences was found in the diameter of the arterial or venular limb.

Discussion

This study shows that in IDDM patients with clinical signs of neuropathy, both baseline arteriovenous shunt flow as well as nutritive capillary blood flow in the foot are increased, opposing the steal hypothesis and supporting the haemodynamic hypothesis. The decrease in skin blood flow normally seen during sympathetic stimulation was smaller in the neuropathic patients. The reaction to postural changes was not different between diabetic patients with and without neuropathy, suggesting a more local reflex.

The patients have been carefully selected from a database of 1054 patients of the outclinical department of an university hospital, on the presence or absence of (autonomic) neuropathy. Since we wanted to be as certain as possible that neuropathy was completely absent, we had to accept some incomplete matching. Consequently patients with neuropathy had a longer diabetes duration, a higher systolic blood pressure and more often microvascular complications. Therefore the difference in microvascular skin blood flow found in this study, can not be definitely ascribed to sympathetic dysfunction alone. The diabetic patients were selected on the basis of absence of vibration sense and lower

leg reflexes. In the neuropathic disability score these two conditions are involved too, which explains the higher score in the neuropathic group. Furthermore differences were found concerning the current perception threshold and vibratory threshold, and cardiovascular autonomic test results. Because of the lack of a clear definition of diabetic neuropathy and absence of a simple, universally accepted test procedure, all the tests were performed in order to define and to quantify the severity of diabetic neuropathy, as carefully as possible [28].

As was found by others, baseline skin temperature and LDF were higher in the diabetic patients with neuropathy [13,29], which is explained by sympathetic hypofunction resulting in opening of the AVA [5,6] and consequently arteriovenous shunting. In agreement with this is the attenuated vasoconstrictor response of the AVA in the neuropathic patients during sympathetic stimulation by an inspiratory gasp [30].

It has been hypothesized that the increase in shunt flow in diabetic patients with neuropathy may compromise capillary nutritive blood flow [8]. This capillary steal phenomenon could explain the disturbed healing potential of a diabetic foot ulcer. Our results are in contradiction with this hypothesis. Flynn et al found no difference in CBV of the toe nail fold between diabetic patients with or without neuropathy, nor did they found an increase in the number of visible capillaries [11]. To detect an increase "volume flow" they measured erythrocyte column width and calculated the product of CBV and erythrocyte column width. In the present study a clear increase in nailfold capillary blood flow was found due to a significantly higher number of functioning capillaries and an increased CBV. In contrast to Flynn et al, we have not found an increase in capillary diameter.

There are a number of differences between the study of Flynn et al and our study. The diabetic patients with and without neuropathy in the study of Flynn et al, did not have a difference in duration of diabetes. NIDDM patients were involved too and none of the diabetic control group had clinical signs of microangiopathy as retinopathy and nephropathy. Flynn et al used the frame to frame technique to measure CBV at five minutes interval, while in this study the cross correlation dual window technique was used, which is a more continuous method to measure capillary blood flow [27].

The significance of our findings is that long-standing raised capillary hyperperfusion

could induce late structural changes, with the ultimate loss of microvascular function and consequently relative underperfusion: the haemodynamic hypothesis [31,32]. Recently Sandeman et al found nailfold capillary hypertension early in the course of diabetes, which may be influenced by changes in metabolic control [33]. The increase in capillary flow due to sympathetic denervation without a change in capillary diameter, which we found results in an increased capillary pressure and may in parallel accelerate intrinsic microvascular functional abnormalities, which can result in the well-known reduced healing potential of the skin following minor trauma.

However, some limitations should be applied to our study results. Skin temperature in the neuropathic group was significantly increased. CBV is positively correlated to skin temperature [10]. Therefore it is unclear if the capillary flow is increased appropriately for the increase in skin temperature. Jörneskog et al found a significant decrease in ratio between capillary and total (LDF) microcirculation in the foot skin of diabetic patients [34], but in their patient groups no increase in baseline LDF was found. Furthermore, in diabetic patients impaired vascular reactivity and limitation of skin microcirculation hyperaemia is found [35]. The latter was also the case in our diabetic population: the percentage LDF increase after suprasystolic toe pressure, was significant lower in both groups of diabetic patients, compared to the healthy controls (data not shown).

No significant difference in postural vasoconstriction response was found between the groups under study. There was an obvious variation in response, as can be seen from the minimum and maximum values. A marked overlap in postural vasoconstriction between normals and patients with diabetic and other neuropathies was reported also by Moy et al [14]. In a previous study we showed that postural vasoconstriction still occurred in the fifth finger after blocking the ulnar nerve [10]. Our results once more suggest that this response is mainly mediated by local neurogenic and/or myogenic mechanisms, only partially supplemented by a central component [17]. Disturbances of this response in diabetic patients do not seem to result from diabetic neuropathy alone, which is in contradiction with other studies [13].

In conclusion: in IDDM patients with neuropathy an increase in anastomotic shunt flow as well as nutritive skin blood flow was found. This contradicts with capillary steal and supports the haemodynamic hypothesis. No difference was observed in the postural

vasoconstriction response between normals and patients with and without diabetic neuropathy, suggesting that this is a local reflex.

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Chapter 10

Summary and conclusions

Summary

Chapter 1.

A striking phenomenon in the neuropathic diabetic foot is the increase in skin blood flow, especially in the dependent position. Despite this increase in peripheral blood flow ulceration may develop often with major healing problems. Sympathetic denervation of arteriovenous anastomoses (AVA) causes hyperaemia of the foot skin. Parallel to the AVA nutritive capillaries are present, which are important in the healing process. It is hypothesized that the increase in AVA flow results in a decrease of capillary blood flow, the so called capillary steal phenomenon.

Chapter 2.

Adequately proven methods to select and to quantify diabetic peripheral neuropathy are lacking. Therefore standardized clinical examination and two sensory perception test devices were compared. Detailed clinical examination, vibration perception threshold (Vibrameter^{R}) and current perception threshold (Neurometer^{R}) of the foot were compared between healthy controls, diabetic patients without and with neuropathy and patients with more than 20 years of diabetes duration. Because of high variability and poor reproducibility, the quantitative sensory threshold measurements are of limited value. Detailed clinical examination seems best suited and was fairly reproducible.

Chapter 3.

To detect diabetic autonomic neuropathy, four cardiovascular reflex tests (forced breathing, standing up, Valsalva manoeuvre and sustained handgrip) are most commonly used. Because much time is required to elaborate the test results, an automated computerized method using a Finapres was developed. With this device, heart rate and blood pressure can be recorded continuously from a finger. The computer program is based on

a timeclock and enables to perform the tests with the calculations within 25 min. The reproducibility of the test results based on heart rate variability showed equal results when compared with the conventional method using an ECG. Age- and sex-dependent normal values of the seven parameters were determined in 124 subjects aged 20 - 90 years. In 10 patients with long-standing (14 - 50 years) complicated diabetes four or more abnormal test parameters were found.

Chapter 4.

Blood flow through AVA are controlled by sympathetic nerve endings. Sympathetic stimulation results in constriction of the AVA and a decrease in total skin blood flow. Sympathetic skin vasomotor reflexes can be tested by laser Doppler fluxmetry (LDF). However, the limited reproducibility and the possible age-dependency of the LDF test results are a matter of concern. In 63 healthy volunteers, two sympathetic stimulation test, distant cooling and inspiratory gasp, were evaluated. No age or sex-dependency of LDF measurements at the big toe were found. The vasoconstriction during distant cooling was too variable (percentage LDF decrease: mean $\pm$ SE, 0.7 $\pm$ 5.3 %) to use in clinical studies. The vascular reaction to inspiratory gasp was more uniform (percentage LDF decrease: 46.5 $\pm$ 3.1 %). The short-term reproducibility of this test was not improved when a thermostatically controlled LDF probe holder at 36^0 was used.

Chapter 5.

To improve the reproducibility of the inspiratory gasp test, the tests were performed under standardized respiratory conditions, by using a spirometer. In 19 healthy controls, the largest decrease in LDF was found when the gasp started end-expiratory and was performed as fast as possible. Continuously sucking negative mouth pressure was followed by a more pronounced vasoconstriction, compared to an inspiratory gasp. Unfortunately standardization of the gasp did not improve reproducibility.

Chapter 6.

Two instruments for LDF measurements were compared; a diode laser (Diodopp^{R}) and a He-Ne gas laser (Periflux Pf1d^{R}). In 20 healthy volunteers, reproducibility of baseline LDF registration, and of 3 standardized provocation tests (postocclusive reactive hyperaemia, tilting and inspiratory gasp) were evaluated, during simultaneous registration with both devices applied to the foot. No difference in spatial variability was found between the instruments. Short-term and long-term reproducibility were better for the Diodopp registrations, but only occasionally the differences reached statistical significance. The registration of the hyperaemic response however, was less pronounced using Diodopp. It was concluded that both LDF devices were equal valuable.

Chapter 7.

Skin microcirculation of the fifth finger was studied before and after denervation by ulnar nerve blockade and compared with skin blood flow of the second finger. After blockade skin temperature and baseline LDF of the fifth finger increased significantly, while the vasoconstrictive response to inspiratory gasp was abolished; both signs of an increase in AVA blood flow. After ulnar blockade the postural vasoconstriction reflex was still present, suggestive for the attribution of local regulatory mechanisms in the changes after tilting. With television capillaroscopy of the nailfold, a significant increase in capillary blood flow of the fifth finger was measured after ulnar nerve blockade. This combined increase in nutritive blood flow as well as AVA blood flow in the sympathetically denervated skin microcirculation are in contradiction with a capillary steal phenomenon.

Chapter 8.

A dBase III program was developed to monitor the diabetic care process of the outpatient clinic of the University Hospital Nijmegen, with special emphasis on foot complications. Diabetic foot problems were the most common (30.4%) long-term complication. Neurological abnormalities such as absence of vibration sense of toes and ankles and lower leg reflexes were more frequent (26.9%) than vascular disturbances (3.8%). In

6.3% of the registered patients a foot ulcer was present during the survey or in the past. From this data base patients were selected for the study described in chapter 9.

Chapter 9.

Skin microcirculation tests were performed in 40 patients with insulin dependent diabetes mellitus and 20 healthy volunteers, all without macroangiopathy. Of the diabetic patients 20 had overt (autonomic) neuropathy and 20 had no clinical signs of neuropathy. Skin temperature and baseline LDF were significantly higher in the patients with neuropathy as compared with the patients without neuropathy and to the healthy volunteers. The smallest LDF decrease during inspiratory gasp was obtained when neuropathy was present. No difference in the postural vasoconstrictor response was seen between the groups. Television capillaroscopy of the nailfold of the left big toe revealed an increased capillary blood flow in the neuropathic group. The finding of an increased AVA blood flow in combination with a capillary overperfusion contradicts the capillary steal phenomenon.

Conclusion

A non-healing neuropathic ulcer of a IDDM patient without peripheral vascular disease of the lower legs, is not explained by a capillary steal phenomenon. In a model (ulnar nerve blockade) and in a carefully selected diabetic population with (autonomic) neuropathy, an increase in arteriovenous *as well as* capillary blood flow was found.
Some precautions should be made in the interpretation of these results. After ulnar nerve blockade and in the IDDM patient group with neuropathy an increase in skin temperature was found. Because capillary blood flow is positively correlated to skin temperature, it remains possible that the increase in nutritive blood flow is not appropriate for the increase in the metabolism, due to the higher skin temperature. Furthermore, it has been demonstrated, that diabetic patients have an impaired vascular reactivity and limitation of skin microcirculation hyperaemia after skin injury or ischaemia due to local

pressure. Under these circumstances skin blood flow is inadequate.

Our findings of an increase in arteriovenous shunt flow and a raised nutritive perfusion support the haemodynamic hypothesis. An increase in capillary blood flow and capillary pressure may induce structural changes of the capillaries and impairment of microvascular function, which consequently causes relative underperfusion of the skin, resulting in a healing problem of a neuropathic ulcer.

Although an increase in foot skin blood flow is especially visible in the dependent position of the lower extremities, no disturbances in posturally induced vasoconstriction of the microcirculation could be demonstrated in the diabetic neuropathic foot. Also, after blockade of the ulnar nerve, no impairment in skin microcirculatory response of the fifth finger was found when lowering the arm. Therefore, the postural vasoconstriction response is mainly mediated by local neurogenic and/or myogenic mechanisms, only partially supplemented by a central component. Disturbances of this response in diabetic patients do not seem to be the result of diabetic neuropathy alone.

Hoofdstuk 11

Samenvatting en conclusies

Samenvatting

Hoofdstuk 1.

De voeten van diabetes patiënten met neuropathie zijn vaak warm en rood, vooral in staande houding of bij afhangen van de benen. Ondanks deze toename in doorbloeding, ontstaan er nogal eens ulceraties met een slechte genezingstendens. Verantwoordelijk voor de toename in huiddoorbloeding zijn, de door sympatische denervatie wijd openstaande arterioveneuze anastomosen (AVA). Parallel geschakeld aan deze AVA zijn de voedingscapillairen, die zorg dragen voor de voeding van de huid en het bevorderen van het genezingsproces. Verondersteld wordt dat de toename in doorstroming door de AVA ten koste gaat van de doorbloeding van de capillairen, het zogenaamde "capillary steal" fenomeen, resulterend in een gestoord genezingsproces.

Hoofdstuk 2.

Voor het selecteren van patiënten met diabetische neuropathie en het kwantificeren ervan ontbreekt een gouden standaard. Vandaar dat de resultaten van een gestandaardiseerde klinische scoringslijst en van twee sensorische tests (bepalingen van de gevoelsdrempel voor vibratiezin [Vibrameter^{R}] en wissel spanning [Neurometer^{R}]) werden vergeleken bij gezonde vrijwilligers, diabetes patiënten met en zonder klinische neuropathie, en bij patiënten met een diabetes duur van meer dan 20 jaar.
Gezien de grote variatie en matige reproduceerbaarheid is de waarde van de tests met beide instrumenten beperkt. Een nauwgezet, gestandaardiseerd klinische onderzoek op de aanwezigheid van neuropathie lijkt het meest geschikt en in ieder geval goed reproduceerbaar.

Hoofdstuk 3.

Voor het opsporen van diabetische autonome neuropathie worden meestal 4 cardiovasculaire reflex tests (geforceerd ademhalen, gaan staan, Valsalva blazen, en isometrische

spierkracht) gebruikt. Daar het uitvoeren en berekenen van de testresultaten veel tijd kost, werd een computerprogramma ontwikkeld, waarbij gebruik gemaakt werd van een Finapres apparaat. Hiermee kan op een niet invasieve, continue wijze de bloeddruk en hartslag gemeten worden aan een vinger. Het computerprogramma is gebaseerd op een tijdklok en maakt het mogelijk de tests uit te voeren en de resultaten te berekenen binnen 25 minuten. De reproduceerbaarheid van deze geautomatiseerde methode is ongeveer gelijk aan de conventionele methode, waarbij gebruik gemaakt wordt van een ECG apparaat en kwikmanometer. Normaal waarden voor leeftijd en geslacht werden verkregen bij 124 gezonde vrijwilligers. Bij 10 patiënten met langdurig diabetes (14 - 50 jaar) en tekenen van lange termijn complicaties werd gevonden dat er 4 of meer van de 7 testparameters afwijkend waren.

Hoofdstuk 4.

De doorstroming van de AVA wordt geregeld door sympatisch zenuwweefsel. Stimulatie van het sympatische zenuwstelsel resulteerde in constrictie van de AVA. Sympatische reflexen die de huiddoorbloeding reguleren, kunnen worden getest gebruikmakend van laser Doppler fluxmetrie (LDF) voor het meten van de huiddoorstroming. De matige reproduceerbaarheid en mogelijke leeftijdsafhankelijkheid van de LDF tests is echter een probleem. Bij 63 gezonde vrijwilligers werden twee sympaticus stimulatie tests, afstandskoeling en diep inademen, geëvalueerd. De LDF metingen werden verricht aan de grote teen. Door ons werd geen leeftijd- of geslachtsafhankelijkheid van de LDF testresultaten gevonden. De LDF veranderingen tijdens afstandskoeling waren te divers (procentuele LDF afname (gemiddelde $\pm$ SE) 0,7 $\pm$ 5,3 %) om toepasbaar te zijn in klinische studies. De diepe inademingstest liet een meer uniforme response zien (procentuele LDF afname: 46,5 $\pm$ 3,1 %). De korte termijn reproduceerbaarheid van deze test kon echter niet verbeterd worden door gebruik te maken van een element dat de huid ter plaatse van de LDF meting verwarmt tot 36^0 C, ter standaardisering van de LDF uitgangswaarde.

Hoofdstuk 5.

Voor het verbeteren van de reproduceerbaarheid van de diepe inademingstest, werd een spirometer gebruikt om de ademhaling te standaardiseren. In 19 gezonde vrijwilligers werd de grootste LDF afname geregistreerd als de inademing eind-expiratoir werd gestart en zo snel mogelijk werd uitgevoerd. Continu negatieve drukzuigen gaf echter de grootste afname in huiddoorbloeding. Helaas gaf standaardisatie van de inademingstest geen verbetering van de reproduceerbaarheid.

Hoofdstuk 6.

In deze studie werden twee instrumenten voor LDF met elkaar vergeleken; een diode laser (Diodopp^{R}) en een Ne-He gas laser (Periflux Pf1d^{R}). Bij 20 gezonde vrijwilligers werd de reproduceerbaarheid nagegaan van basale LDF metingen en 3 provocatie tests (post occlusie hyperemie test, passief gaan staan en diep inademen) tijdens gelijktijdig meten met beide instrumenten aan de voet. Er werd geen verschil gevonden in reproduceerbaarheid van de basale LDF metingen op aangrenzende lokaties. De korte - en lange termijn reproduceerbaarheid van de provocatie tests was in het algemeen beter met de Diodopp, maar slecht zelden werden er significante verschillen gevonden. De hyperemische response werd minder duidelijk geregistreerd door de Diodopp. Geconcludeerd werd dat beide LDF apparaten in het gebruik vergelijkbare resultaten geven.

Hoofdstuk 7.

Huiddoorbloedingsmetingen van de vijfde vinger werden verricht voor en na blokkade van de nervus ulnaris en vergeleken met de tweede vinger. Na zenuwblokkade steeg de huidtemperatuur en basale LDF van de pink significant, terwijl er geen afname meer was in LDF tijdens de diep inademingstest. Deze resultaten worden verklaard door een toename in doorstroming door de AVA. Tevens werd na blokkade gevonden dat de LDF blijft afnemen bij passief gaan staan, hetgeen suggereert dat deze reflex tot stand komt door lokale regulatie mechanismen. Met behulp van een capillairmicroscoop werd de doorbloeding van het nagelbed bestudeerd. Gevonden werd dat na blokkade van de nervus ulnaris de capillaire doorbloeding van de vijfde vinger ook toenam. De toename

van zowel de AVA als capillaire doorstroming na zenuwblokkade, pleit tegen een "capillary steal" fenomeen.

Hoofdstuk 8.

Met behulp van een dBase III programma werd de diabetes polikliniek van het Academisch Ziekenhuis Nijmegen St Radboud in kaart gebracht, waarbij er bijzondere aandacht was voor voetproblemen. Van de lange termijn complicaties van diabetes, was het diabetisch voetprobleem het meest frequent (30,4%). Vooral neurologische afwijkingen als afwezige vibratiezin aan enkels en tenen en a(hypo)reflexie kwamen frequent (26,9 %) voor. Slecht bij 3,8% van de patiënten waren er tekenen van perifeer vaatlijden. Bij 6,3% van de patiënten was er een voetulcus aanwezig of had de patiënt dat in het verleden gehad. Met behulp van dit bestand werden de patiënten geselecteerd voor de studie beschreven in hoofdstuk 9.

Hoofdstuk 9.

In 40 patiënten met insuline afhankelijke diabetes en 20 gezonde vrijwilligers, allen zonder tekenen van macro-angiopathie, werd de huiddoorbloeding gemeten onder verschillende condities. Van de diabetes patiënten hadden 20 duidelijke tekenen van (autonome) neuropathie. De huidtemperatuur en de basale LDF waren significant hoger bij de patiënten met neuropathie, in vergelijking met de andere diabetespatiënten en met de vrijwilligers. De diepe inademingstest gaf de geringste vasoconstrictie in geval er neuropathie aanwezig was. Geen verschillen werden er gevonden tussen de drie groepen in LDF response tijdens passief gaan staan. De gemeten capillaire doorstroming door het nagelbed van de eerste teen was in de groep met neuropathie toegenomen. Gezien de toename in AVA en in capillaire doorstroming werden er dus geen aanwijzingen gevonden voor een "capillary steal" fenomeen.

Conclusies

De slechte genezingstendens van een neuropathisch voetulcus bij patienten met diabetes mellitus zonder tekenen van macroangiopathie wordt niet verklaard door het bestaan van een "capillary steal" fenomeen Zowel in een experimenteel model (ulnaris blokkade) als bij zorgvuldig geselecteerde diabetes patienten met (autonome) neuropathie, werd zowel een toename in arterioveneuze shunt flow, als in capillaire doorstroming gevonden
De hogere huidtemperatuur ten gevolge van de toegenomen shuntflow maakt wel dat enige terughoudendheid geboden is. Een hogere huidtemperatuur leidt tot een actiever metabolisme van de huid, waardoor de capillaire doorstroming moet toenemen om aan de grotere behoefte te voldoen. Het is niet uitgesloten dat de toename in capillaire doorstroming geen gelijke tred houdt met de toename in huidtemperatuur.
Daarnaast is aangetoond dat de maximale, reactieve toename in huiddoorbloeding bij patienten met diabetes mellitus beperkt is Na een trauma of wegvallen lokale druk kan de huiddoorstroming onvoldoende toenemen, wat leidt tot een inadequate perfusie van de voethuid onder deze omstandigheden
De toename van zowel de arterioveneuze als capillaire doorstroming, past bij de haemodynamische hypothese, volgens welke een toename in capillaire doorstroming en intracapillaire druk leiden tot microvasculaire sclerose en verstoring van de functie van de circulatie bij het genezingsproces van een neuropathisch ulcus
Ofschoon de toename in arterioveneuze huiddoorbloeding vooral zichtbaar is in staande houding of bij afhangende ledematen, kon dit niet worden aangetoond bij patienten met een diabetische neuropathische voet. Ook na blokkade van de nervus ulnaris werd er bij afhangende arm geen significante toename in huiddoorbloeding gemeten aan de vijfde vinger De regulatie in huiddoorbloeding bij veranderde houding wordt waarschijnlijk niet primair geregeld door het perifere zenuwstelsel, maar door myogene factoren en/of lokale axon reflexen.

List of additional papers concerning the diabetic foot or methods described in this thesis.

EPHEDRINE IMPROVES MICROCIRCULATION IN THE DIABETIC NEUROPATHIC FOOT.

Wollersheim H, Netten PM, Lutterman JA, Lenders JWM.

Angiology 1989; **40**: 1030-1034.

EVALUATION OF INFECTIOUS DIABETIC FOOT COMPLICATIONS WITH INDIUM-111-LABELED HUMAN NONSPECIFIC IMMUNOGLOBULIN G.

Oyen WJG, Netten PM, Lemmens AM, Claessens AMJ, Lutterman JA, Vliet van der JA, Goris RJA, Meer van der JWM, Corstens FHM.

Journal of Nuclear Medicine 1992; **33**: 1330-1336.

ANTIBIOTISCHE THERAPIE BIJ DIABETISCHE VOETULCERA.

Sauerwein RW, Netten PM, Koopmans PP.

Nederlands Tijdschrift voor Geneeskunde 1994; **138**: 557-560.

AUTONOMIC NEUROPATHY IN DIABETIC PATIENTS WITH END-STAGE RENAL DISEASE, TESTED WITH A FINAPRES DEVICE.

Netten PM, Arend den JACJ, Olderiekerink EAM, Hem van der LG, Lutterman JA, Thien Th.

Homeostasis in Health and Disease, in press

AUTONOMIC DYSFUNCTION IN PARKINSON'S DISEASE, TESTED WITH A COMPUTERIZED METHOD, USING A FINAPRES DEVICE.

Netten PM, Vos de K, Horstink MWIM, Hoefnagels WHL.

Clinical Autonomic Research, 1995; **5:** 85-89.

MICROCIRCULATION IN THE FOOTSOLE AS A FUNCTION OF MECHANICAL PRESSURE.

Meinders MJ, Lange de A, Netten PM, Wollersheim H, Lutterman JA.

Clinical Biomechanics, in press

DISTURBED PRESSURE-INDUCED REACTIVE HYPERAEMIA IN THE FOOTSOLE OF DIABETES.

Meinders MJ, Netten PM, Lange A, Wollersheim H, Lutterman JA.

Submitted

Dankwoord

Het schrijven van een proefschrift is voor een belangrijk deel het resultaat van het coördineren van het werk van velen. Alleen door een goede samenwerking ontstaan creatieve ideeën en oplossingen. Vandaar dat het op zijn plaats is velen te bedanken voor hun inzet.

Op de eerste plaats de meer dan 400 proefpersonen, die met veel geduld bereid waren de verschillende tests te ondergaan, onder vaak vervelende "standaard condities".

Prof. Dr. Ab van 't Laar voor zijn inspanningen bij de start van dit onderzoek en zijn kritisch commentaar wanneer te veel enthousiasme dreigde.

De studenten Christien Segeren, Hans Boots, Bas Bredie, Lodewijk Keeris, Paulien van de Broek, Erik van der Heijden, Peggy du Buf-Vereijken, Marieke Lucassen, Katinka de Vos en Marjan Meinders, die in het kader van een wetenschappelijke stage of een afstudeerstage gezondheidswetenschappen, belangrijke onderdelen van het beschreven onderzoek verrichten. De "enthousiaste en leerzame begeleiding" was het resultaat van een plezierige samenwerking met hen. Ook Jan Willem Meijer en Marian Scheepers moeten hier genoemd worden. Onder begeleiding van collega Cees Tack hebben zij waardevol onderzoek verricht naar de waarde van sensorische testmethoden ter detectie van diabetische neuropathie.

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In goede samenwerking met Prof. Dr. Willibrord Hoefnagels, klinisch geriater, kon het geautomatiseerde computerprogramma voor cardiovasculaire reflextests ontwikkeld worden.

Joost den Arend, zonder wiens inzet al het computerwerk zeker niet gelukt zou zijn en die telkenmale bereid was om de "harde schijven" opnieuw te installeren.

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Mieke van Bergen, Lammy Elving, Paul Smits, Cees Tack, Petra van de Ven en Janny

de Best voor het kritisch beluisteren van alle onderzoeksresultaten en de perikelen rondom het onderzoek.
De leden van de hypertensie en circulatie werkgroep voor alle kritische kanttekeningen.

Mijn ouders, die er op wezen dat er "met je best te doen" veel te bereiken is.
Voor David en Felix zal het wennen zijn nu "het boek" klaar is. Voortaan zullen we tekenpapier moeten kopen. Tot nu toe stond op de achterkant van jullie creatieve uitingen een met rode pen gecorrigeerd onderdeel van het proefschrift.
En tot slot Yvonne, op jouw is de tekst uit een lied van Frans Halsema zeer toepasselijk: *"als er iemand op de wereld is, die mij bevrijden kan van franje en vernis, dan ben jij het wel."*

Curriculum vitae

Paetrick Netten werd op 4 april 1957 te Eindhoven geboren. In 1975 behaalde hij het VWO diploma aan het Dominicus College te Nijmegen. In dat zelfde jaar werd een aanvang gemaakt met de studie Geneeskunde aan de Katholieke Universiteit Nijmegen (Kandidaats examen (cum laude) 1977; Doctoraal examen (cum laude) 1980), alwaar in 1982 het artsexamen werd behaald. Aansluitend was hij tot 1985 als internist in opleiding werkzaam op de afdeling interne geneeskunde in het Groot Ziekengasthuis te 's-Hertogenbosch (Opleiders: Dr. J.B. Lips en Dr. J.L.J. Jansen), waarna de specialisatie tot internist werd afgerond in het Academisch Ziekenhuis te Nijmegen (opleider; Prof. Dr. A. van 't Laar).

Van 1987 tot 1993 was hij werkzaam op de afdeling algemeen interne geneeskunde en nauw betrokken bij de diabetes zorg (werkgroepleider: Dr J.A. Lutterman). In de periode 1990 tot 1993 werd dankzij een subsidie van het Diabetes Fonds Nederland het onderzoek verricht dat uiteindelijk resulteerde in dit proefschrift. Sinds 1993 is hij werkzaam als internist, met als aandachtsgebied diabetes mellitus, in het Bosch Medicentrum, locatie Groot Ziekengasthuis te 's-Hertogenbosch.

STELLINGEN

behorend bij het proefschrift

The influence of sympathetic failure on the skin microcirculation of the diabetic neuropathic foot.

Paetrick Netten

20 september 1995

1. De toename in voethuiddoorbloeding in staande houding zoals die kan voorkomen bij patiënten met diabetes mellitus is niet het gevolg van een neuropathie.

2. In de voethuid van een patiënt met een diabetische neuropathie is zowel de arterioveneuze als capillaire doorstroming toegenomen.

3. De kwantitatieve methoden, voor het vaststellen van een sensorische neuropathie, gebruikmakend van een vibrameter of neurometer, zijn van beperkte waarde, gezien de grote variabiliteit en matige reproduceerbaarheid.

4. Het zou goed zijn als er uniforme, internationaal geaccepteerde, klinische scoringslijsten ontwikkeld zouden worden ten behoeve van het beschrijven van de ernst van diabetische neuropathie.

5. Het is nog onvoldoende duidelijk wanneer en hoe een diabetisch voetulcus met antibiotica moet worden behandeld.
(RW Sauerwein, PM Netten, PP Koopmans. Ned Tijdschr Geneeskd 1994; 138: 557-560).

6. Hypomagnesiëmie door cisplatinum kan worden voorkomen door toediening van hoge doseringen $MgCl_2$ tijdens de cisplatinum infusie.
(PM Netten, PHM de Mulder, AG Theeuwes, JL Willems, BEM Kohler, DJTh Wagener. Ann Oncology 1990; 1: 369-372).

7. Intraveneus toedienen van vitamine K is zinloos en niet ongevaarlijk.
(RWMM Jansen, RC Rietbroek, PM Netten. Ned Tijdschr Geneeskd 1990; 134: 1673-1675).

8. Met de voet in de hand voorkomt men veel trammelant.

9. Voor de uitvoering van het plan Biesheuvel is uitbreiding van de opleidingscapaciteit voor medisch specialisten noodzakelijk.

10. Suma-worstelaars zijn geen prototypen voor patiënten met het syndroom X.

11. Het telkenmalen vernieuwen van "software" voor PC's, dient op de eerste plaats commerciële belangen.

12. Een ziekenhuis-fusie zonder ruzie, is een illusie.